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Cytological Diagnosis of Gastric Cancer

By

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Studies on the cytological diagnosis of cancer have been undertaken since many decades ago.[1-4] In 1928, Papanicolaou discovered some cells specific to cancer in the vaginal smears from patients with uterine cancer and published a systematic report on them;[5] especially, the book entitled "Diagnosis of Uterine Cancer by Vaginal Smear" published under the joint authorship of Papanicolaou and Traut in 1943[6] which drew the attention of many investigators in the world which are concerned with the problem of cancer and after that the subject speedily became a problem of the present day.

In gastric cancer cases, Rosenbach[7] first reported on cancer cells and cancerous tissue particles in the gastric contents in 1882. Since Papanicolaou, in 1946, demonstrated the diagnostic value of exfoliated cells from the cancerous tissues, taking not only the vaginal secretion of uterine cancer cases but also the gastric juice of gastric cancer cases into account,[8] the reports on cytological diagnosis of gastric cancer are increasing at present day.[9-37] In Japan, excellent studies were reported by Ayabe,[38] Oda,[39] Yugawa,[40] Tsuda[41] and co-workers, and others.[42-46] The accuracy of the cytologically correct diagnosis in gastric cancer is ranging from 22.0[8] to 93.8 per cent[29] on reviewing the outstanding literatures in the world.

In this paper, the results of cytological diagnosis of gastric cancer applied to patients in the Medical Clinic of Prof. T. Kurokawa, Tohoku University, are mentioned. The analysis is limited to those cases in which material was collected from the stomach by the gastric abrasive balloon, and includes a comparison with location, size and pathologic types of gastric cancer, acidity of the gastric juice, biochemical cancer tests, and so on.

Experimental

Method and Material

The cytological material used in this investigation was obtained solely by the abrasive balloon method, the apparatus for which consists of a Miller-Abbott's double-lumen rubber tube with a thin rubber balloon (a condom which was cut at both ends shortly) fastened to the distal end. The balloon is encased in a wide-meshed silk netting (Figs. 1 and 2). One lumen of the tube is used for inflating the balloon, the other for aspirating gastric contents. With the patient in a sitting position the tip of the tube assembly placed behind the epiglottis. With repeated degultition the tube is carried into the stomach. The total gastric contents are aspirated and discarded. Under fluoroscopy the tip of the tube is send to the pyloric area of the stomach, the balloon is inflated and the tube is drawn up until the inflated balloon abut against the cardioesophageal junction. This procedure is usually repeated three times. In order to expedite antral abrasion the balloon in antrum is reinflated and redeflated and abrasion of the antrum is performed several times. No more than 70 cc. of air are introduced in this area. The balloon is then deflated, withdrawn from the patient and reinflated. A part of adherent mucus and tissue on the surface of the balloon is smeared on slides, fixed and stained according to the Papanicolaou's method and the May-Grünwald-Giemsa's method, and the other part of that is used for the phase-contrast-microscopic method.

The patients are given nothing after midnight of the day before the test is to be performed. Patients known to be constricted are restricted to fluid for the 24 hours prior to the nine-hour fast before the test. Before and if necessary during the test the gastric contents are respirated through one lumen of the tube and discarded, and swallowing of saliva, nasal secretions and sputum was strictly prohibited.

Cytological findings are classified in this clinic on the basis of the following schema; Class I—absence of atypical or abnormal cells; Class II—atypical cells present but no evidence of malignancy; Class III—cells present giving a suspicion of malignancy; Class IV—fairly conclusive evidence of a malignant neoplasm; Class V—conclusive evidence of a malignant neoplasm. The findings of Class I and II are judged as negative, Class III as suspicious and Class IV and V as positive for cancer.

Results

1. Over-all results of cytological diagnosis

Of the 831 patients studied, gastric cancer was proved at operation and autopsy or was diagnosed as positive for cancer on the basis of definite

clinical findings in 303. The remaining 528 were patients with other diagnosis.

The over-all results shown in Table I indicate that in the cancer group 90.4 per cent of the cases correctly reported as cytologically positive

TABLE I

Over-all Results of Cytological Diagnosis in 831 Cases

	No. of cases	Cytology				
		Negative		Suspicious	Positive	
		I	II	III	IV	V
Cancer	303	8	21	16	58	200
		9.6%		90.4%		
No cancer	528	155	358	5	6	4
		97.2%		2.8%		
Total	831	163	379	21	64	204

or suspicious, 9.6 per cent failed to exhibite cells positive or suspicious for cancer and in the non-cancerous group 97.2 per cent were correctly interpreted as negative, 2.8 per cent were incorrectly read as suspicious or positive for cancer.

2. The relation between location of lesions and results of cytological dianosis (Table II)

In the cancer group the accuracy of the correct diagnosis was 98.9 per cent in the neoplasms of the cardia-fornix, 89.6 per cent of the corpus and 82.1 per cent of the antrum-pylorus; the accuracy was higher in the neoplasms which located in the more cranial site of the stomach.

In the non-cancerous group the frequency of the cytologically suspicious or positive reports was 4.9 per cent in the lesions of the cardia-fornix, 2.6 per cent of the corpus and 0.9 per cent of the antrum-pylorus; the frequency of the incorrect diagnosis was higher in the lesions which located in the more cranial site of the stomach.

3. The relation between size of neoplasms and results of cytological diagnosis

The maximum diameter of the neoplasm in the stomach which had undergone a gastric resectomy was measured and the results were studied in relation with results of cytological diagnosis (Tables III and IV).

The accuracy of the correct diagnosis was 68.4 per cent in the neoplasms of the less than 5.0 cm. in diameter, 91.8 per cent of the 5.0 to 10.0 cm. and 94.8 per cent of the above 10.1 cm.; the accuracy was lower in the neoplasms which have the smaller size in maximum diameter. In

TABLE II

The Relation between Location of Lesions and Results of Cytological Diagnosis

	Location	No. of cases	Cytology				
			Negative		Suspicious	Positive	
			I	II	III	IV	V
Cancer	Cardia-fornix	90	0	1 (1.1%)	0	10	79 (98.9%)
	Corpus	135	5	9 (10.4%)	10	29	82 (89.6%)
	Antrum-pylorus	78	3	11 (17.9%)	6	19	39 (82.1%)
	Total	303	8	21	16	58	200
No cancer	Cardia-fornix	144	38	99 (95.1%)	2	3	2 (4.9%)
	Corpus	270	82	181 (97.4%)	3	3	1 (2.6%)
	Antrum-pylorus	144	35	78 (99.1%)	0	0	1 (0.9%)
	Total	528	155	358	5	6	4

TABLE III

The interrelation of size of Neoplasms and Results of Cytological Tests (1)

Muximum diameter	No. of case	Cytology				
		Negative		Suspicious	Positive	
		I	II	III	IV	V
0—— 5.0 cm.	47	5	10 (31.6%)	3	10	19 (68.4%)
5.1——10.0 cm.	85	0	7 (8.2%)	3	16	59 (91.8%)
Above 10.1 cm.	35	0	2 (5.7%)	0	7	26 (94.3%)
Total	167	5	19	6	33	104

TABLE IV

The interrelation of size of Neoplasms and Results of Cytological Tests (2)

Location	Muximum diameter	Total	Cytology					Accuracy
			Negative		Suspicious	Positive		
			I	II	III	IV	V	
Cardia-fornix	0—— 5.0 cm.	7	0	0	0	1	6	100.0%
	5.1——10.0 cm.	28	0	1	0	4	23	96.4%
	Above 10.1 cm.	10	0	0	0	1	9	100.0%
Corpus	0—— 5.0 cm.	22	3	5	2	3	9	63.6%
	5.1——10.0 cm.	32	0	3	1	8	20	90.6%
	Above 10.1 cm.	13	0	0	0	5	8	100.0%
Antrum-pylorus	0—— 5.0 cm.	18	2	5	1	6	4	61.1%
	5.1——10.0 cm.	25	0	3	2	4	16	88.0%
	Above 10.1 cm.	12	0	2	0	1	9	83.3%
Total		167	5	19	6	33	104	85.6%

TABLE V

The interrelation of macroscopic Types of Neoplasms (by Borrmann-Konjetzny's Classification) and Cytological Tests Results

Borrmann-konjetzny's classification	No. of cases	Number of positive cases	Accuracy
Type I	20	18	90.0%
Type II	73	54	74.0%
Type III	63	58	92.1%
Type IV	21	21	100.0%
Total	187	161	85.6%

the neoplasms of the cardia-fornix, however, the accuracy was high regardless of size of neoplasm. The increased accuracy may have been attributed to be capable of the sufficient abrasion of the neoplasm in such location. In the neoplasms of the corpus and the antrum-pylorus the accuracy was only the approximate 60 per cent of the neoplasms of the less than 5.0 cm. in diameter.

4. The relation between macroscopic types of neoplasms and results of cytological diagnosis

Macroscopic types of neoplasms in the stomach which had undergone a gastric resectomy were devided into four, according to the macroscopic classification of the neoplsm by Borrmann-Konjetzny[47,48] and the results were studied in relation with results of cytological diagnosis (Table V).

The accuracy of cytological diagnosis was 90.0 per cent in the neoplasms of Type I, 74.0 per cent of Type II, 92.1 per cent of Type III and 100.0 per cent of Type IV; the accuracy was highest in the neoplasms of Type IV, next in those of Type III and Type I, and lowest in those of Type II.

5. The relation between histological types of neoplasms and results of cytological diagnosis (Table VI)

TABLE VI

The interrelation of histological Types of Neoplasms and Cytological Tests Results

Histological types	No. of cases	Cytology					Accuracy
		Negative		Suspicious	Positive		
		I	II	III	IV	V	
Adenocarcinoma	65	4	3	3	17	38	89.2%
Simple carcinoma	44	0	5	3	6	30	88.6%
Scirrhous carcinoma	25	0	4	1	6	14	84.0%
Gelatinous carcinoma	3	0	0	0	0	3	100.0%
Mixed type	6	0	0	0	0	6	100.0%
Total	143	4	12	7	29	91	88.8%

Neoplasms in the stomach which had undergone a gastric resectomy were histologically classified into five types; adenocarcinoma, single carcinoma, scirrhous carcinoma, gelationous carcinoma and the mixed type of the above four.

The accuracy of cytological diagnosis was highest in the neoplasm of mixed and gelationous types (100.0 per cent respectively), followed by adeno- (89.2 per cent), single (88.6 per cent) and scirrhous carcinoma (84.0 per cent) in descending order. It is to be noted that the accuracy was considerably high even in the neoplasms of scirrhous type, to whom the low accuracy has been attributed hitherto.

6. The relation between acidity of the gastric juice and results of cytological diagnosis (Tables VII and VIII)

In the cancer group the accuracy of cytological diagnosis was 94.9 per cent of the 176 anacid cases, 89.7 per cent of the 29 hypoacid cases, 66.7 per cent of the 33 normoacid cases and 77.8 per cent of the 9 hyperacid cases; the accuracy was higher in the cases with the lower degree of the acidity. In the neoplasms of the cardia-fornix, however, the accuracy was generally high, though it was slightly lower in the hyperacid cases.

In the non-cancerous group the frequency of the cytologically in-

TABLE VII

The interrelation of Acidity of the Gastric Juice and Cytological Tests Results

	Acidity	No. of cases	Cytology				
			Negative		Suspicious	Positive	
			I	II	III	IV	V
Cancer	Anacid	176	9		9		158
			5.1%		94.9%		
	Hypoacid	29	3		1		25
			10.3%		89.7%		
	Normoacid	33	11		3		19
			33.7%		66.7%		
	Hyperacid	9	2		2		5
			22.2%		77.8%		
	Total	247	25		15		207
No cancer	Anacid	148	139		4		5
			93.9%		6.1%		
	Hypoacid	83	80		0		3
			96 4%		3.6%		
	Normoacid	123	122		0		1
			99.2%		0.8%		
	Hyperacid	100	99		1		0
			99.0%		1.0%		
	Total	454	440		5		9

correct diagnosis was 6.1 per cent of the 148 anacid cases, 3.6 per cent of the 83 hypoacid cases, 0.8 per cent of the 123 normoacid cases and 1.0 per cent of the 100 hyperacid cases; the frequency of the incorrect diagnosis was higher in the cases with the higher degree of the acidity.

7. Results of the diagnosis by different methods of cytological examination (Table IX)

The accuracy of the correct diagnosis in the cancer group as well as the frequency of the incorrect diagnosis in the non-cancerous group showed scarcely difference whether the phase-contrast-microscopic method (P.C.M.) (Figs. 6, 7, 8, 9, 10 and 11), or the May-Grünwald-Giemsa's method (M.G.G.) (Figs. 3, 4 and 5), or the Papanicolaou's method was used. Any of those methods has its respective merits and demerits and,

TABLE VIII

The interrelation of Acidity of the Gastric Juice and Cytological Tests Results in the Cancerous Cases

Location	Acidity	No. of cases	Cytology			Accuracy
			Negative	Suspicious	Positive	
			I II	III	IV V	
Cardia-fornix	Anacid	35	0	0	35	100.0%
	Hypoacid	12	1	0	11	91.7%
	Normoacid	4	0	0	4	100.0%
	Hyperacid	5	0	0	5	100.0%
	Total	56	1	0	55	98.2%
Corpus	Anacid	87	3	5	79	96.6%
	Hypoacid	12	1	1	10	91.7%
	Normoacid	19	8	2	9	57.9%
	Hyperacid	2	1	1	0	50.0%
	Total	120	13	9	98	89.2%
Antrum-pylorus	Anacid	54	6	4	44	76.3%
	Hypoacid	5	1	0	4	80.0%
	Normoacid	10	3	1	6	70.0%
	Hyperacid	2	1	1	0	50.0%
	Total	71	11	6	54	84.5%
Total		247	25	15	207	89.9%

therefore, it is desirable to make most of the merits of one of them and get experienced to it. Practically, it seems convinent to apply the routinely familiar M.G.G. method.

8. Chronological changes of the results of cytological diagnosis

Results of cytological diagnosis were chronologically divided into five parts at intervals of approximate one year each, as shown in Table X. The accuracy of the correct diagnosis in the cancer group fluctuated between 88 and 98 per cent, but the frequency of the incorrect diagnosis in the non-cancerous group has decreased from ca. 6.5 per cent in the first two periods to 0 per cent in the last two, showing that the liability of getting false-positive results may be reduced by the aquisition of the increased experience of identifying the cells. These results suggest the approximate limits of the accuracy of cytological diagnosis.

9. A comparison of results between biochemical cancer tests and cytological diagnosis (Table XI)

Biochemical cancer tests, that is, the ninhydrin reaction test of the gastric juice[49] and the Matsubara's cancer reaction test[50] were studied in comparison with results of cytological diagnosis applied to the same subjects.

TABLE IX

Results of the Diagnosis by Different Methods of Cytological Examination

	Method	No. of cases	Cytology				
			Negative		Suspicious	Positive	
			I	II	III	IV	V
Cancer	P.C.M.*	293	8	28	21	54	182
			12.3%		87.7%		
	M.G.G.**	259	6	26	21	51	155
			12.4%		87.6%		
	Papanic.***	99	3	15	5	22	54
			18.2%		81.8%		
	Combined****	303	8	21	16	58	200
			9.6%		90.4%		
No cancer	P.C.M.	521	159	348	7	5	2
			97.3%		2.7%		
	M.G.G.	446	124	313	0	6	3
			98.0%		2.2%		
	Papanic.	136	59	72	2	2	1
			96.3%		3.7%		
	Combined	528	155	358	8	6	4
			97.2%		2.8%		

* P.C.M.—Phase-contrast-microscopic method.
** M.G.G.—May-Grunwald-Giemsa's method.
*** Papanic.—Papanicolaou's method.
**** Combined—A combined application of the above three methods.

In the cancer group the accuracy of the correct diagnosis of the ninhydrin reaction test was 70.3 per cent and less satisfactory than that of cytological diagnosis (88.3 per cent), while in the non-cancerous group only 3.4 per cent of the cases showed false-positive reports in the cytological diagnosis, but a much higher percentage (24.4 per cent) of that was obtained by the ninhydrin reaction test. With the Matsubara's cancer reaction test, the similar relation of results was obtained. Thus, it has been ascertained that the cytological diagnosis assures a higher accuracy in both cancer and non-cancerous groups than any of such biochemical cancer tests, that is to say, is of higher reliability.

10. A review of the misdiagnosed cases (Tables XII and XIII)

TABLE X

Chronological Changes of the Results of Cytological Diagnosis

	Method	Accuracy in cancer and error in non-cancer					
		1st period	2nd period	3rd period	4th period	5th period	Over-all period
Cancer	P.C.M.	88.3%	80.0%	100.0%	96.8%	89.6%	87.7%
	M.G.G.	73.9%	91.7%	81.5%	93.8%	88.2%	87.6%
	Papanic.	——	83.0%	82.4%	——	——	82.7%
	Combined	88.3%	91.7%	84.6%	98.5%	90.2%	90.4%
No cancer	P.C.M.	6.2%	6.0%	3.2%	0%	0%	2.7%
	M.G.G.	0%	7.1%	2.8%	0%	0%	2.0%
	Papanic.	——	5.7%	1.5%	——	——	3.7%
	Combined	6.2%	7.1%	3.7%	0%	0%	2.8%

TABLE XI

Comparison of Results between Biochemical Cancer Tests and Cytological Diagnosis

	Method	No. of cases	Cytology		
			Negative	Suspicious	Positive
Cancer	Cytological diagnosis	111	13 / 11.7%	8	90 / 88.3%
	Ninhydrin reaction	111	33 / 29.7%	0	78 / 70.3%
No cancer	Cytological diagnosis	176	170 / 96.6%	0	6 / 3.4%
	Ninhydrin reaction	176	133 / 75.6%	2	41 / 24.4%
Cancer	Cytological diagnosis	79	12 / 15.2%	5	62 / 84.8%
	Matsubara's cancer reaction	79	18 / 22.8%	3	58 / 77.2%
No cancer	Cytological diagnosis	83	75 / 90.4%	4	4 / 9.6%
	Matsubara's cancer reaction	83	53 / 63.9%	7	23 / 36.1%

False-negative reports were obtained in 29 of the 303 (9.6 per cent) cancerous cases and false-positive reports were obtained in 15 of the 528 (2.8 per cent) non-cancerous cases. False-negative results were mainly

TABLE XII

Causes of False Results

Causes	False-positive	False-negative
	29 cases	15 cases
I. Defective technique		
1) Inadequate preparation of patients	2	0
2) Insufficient and inadequate abrasion	14	0
3) Insufficient aspiration of the gastric juice	3	5
4) Defective preparation of specimens	1	1
5) Unskilled identification of cells	3	13
II. Intragastric unfavorable conditions		
1) Overbleeding	1	0
2) Regurgitation of bile into the stomach	1	5
3) Gastric retention and changes of cells by gastric juice	3	4
III. Properties of the tumour itself		
1) Covering with necrotic membranes	3	0
2) Mainly consisting of intramucosal infiltration	11	0

TABLE XIII

False-positive Results by Disease Type

Disease type	No. of cases	Cytology		
		Suspicious	Positive	
		III	IV	V
Gastritis	274	1	2	2
		5 (1.8%)		
Gastric ulcer	168	2	4	1
		7 (4.2%)		
Gastric polyp	17	1	0	1
		2 (1.2%)		
Others	69	1	0	0
		1 (1.4%)		
Total	528	5	6	4
		15 (2.8%)		

attributed to the insufficient and inadequate abrasion and to the intramucosal infiltration of neoplasm, but it is to be noted here that one and three cases respectively had to be attributed to imperfection in preparing the specimen and to the insufficient experience of the identifying the cells.

False-positive results must be in fine attributed to the low skill in identifying the cells, but it must be noted that the degeneration of gastric cells caused by gastric juice and the regurgitated bile into the stomach is sometimes too strong for identifying the cells. As for diseases, such false-poistive results were most frequent in cases with gastric ulcer, followed by cases with gastritis and gastric polyp in the order named. In cases with gastric ulcer, false-positive reports reached 4.2 per cent and since it is the highest in cases with callous ulcer, special caution is required in evaluating such cases.

Reports of the incorrect diagnosis may be reduced in frequency to a certain extent by the aquisition of the increased experience of collection technique and identifying the cells in all probability, for the number of false-positive cases could be drastically reduced as mentioned in 8 above.

Discussion

Till only one decade ago, cytological diagnosis of gastric cancer was regarded as a difficult problem because of the more unfavorable conditions in the stomach under which the cytological examination have to be carried out than in other organs. Pollard *et al.*[14] pointed out the following factor of difficulty in such examinations. 1) Only the very quick and nible fixation can save the cancer cells from destruction by gastric juice. 2) Scirrhous carcinoma is most frequent in gastric cancer, and cancer cells are scarcely exfoliated from such neoplasms. 3) Frequently cells exfoliated and carried down from the nasopharyngeal, the esophageal and the tracheal mucous membranes and food residue lead to the confusion of the microscopic findings. At present, however, the improvement of the collection technique and the aquisition of the increased experience in identifying the cells have enable us to surmount such difficulties in all probability.

Particularly, the abrasive balloon method invented by Panico *et al.*[16] is a very recommendable method, for it is easy and simple in clinical application, cause little pain to patients and enable sampling fresh cancer cells in a quantity, as already described by many authors in Japan and foreign lands.

The accuracy of the cytologically correct diagnosis differs by different techniques for the collection of gastric cells. A general review of the past literature on the subjects gives the values ranging from 22.0[8] to 93.8 per cent.[29] The accuracy obtained by the present authors by the abrasive balloon method was 90.4 per cent of the cancerous cases—a value next to that of Graham (93.8 per cent).[29]

The accuracy differs also by location of neoplasms, the accuracy running up to the high of 93.9 per cent in the neoplasms of the cardiafornix. Such a excellent results is certainly of high significance when it

is recalled that some neoplasms of the cardia have been particularly difficult to diagnose hitherto. The accuracy was, however, lower when neoplasms was located in the antrum-pylorus. This seems to be inevitable inasmuch as the success of tests under this method depends on the adequacy of abrasion.

In the last phase, the cytologically incorrect reports in the non-cancerous group are to be mentioned. The false-positive results have been reported as ammounting to 4 to 11 per cent (Graham), but the much lower percentage of 2.8 per cent was obtained in our study. At any rate, a careful and critical analysis of the false-positive reports will probavely serve in enlarging the scop of utility of cytological diagnosis.

Summary and Conclusions

1. The accuracy of cytological diagnosis of 303 gastric cancers by the abrasive balloon method was 90.4 per cent and the false-positive or suspicious reports of 528 non-cancerous cases were 2.8 per cent.

2. The accuracy was very high (98.9 per cent) in the neoplasms of the cardia-fornix.

3. The results of cytological diagnosis of gastric cancer were mentioned in relation with size and pathologic types of gastric cancer, acidity of the gastric juice and biochemical cancer tests, and so on.

4. The cytological method by the abrasive balloon is a very helpful method as one of the diagnostic tests for gastric cancer, especially serviceable in the cases where cancer is in the cardia-fornix, or the corpus.

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Fig. 1

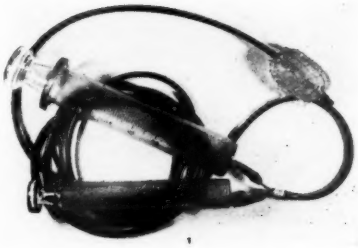
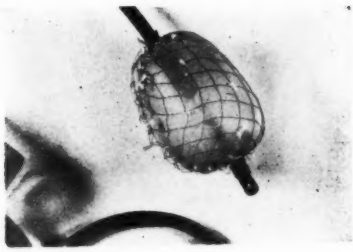


Fig. 2



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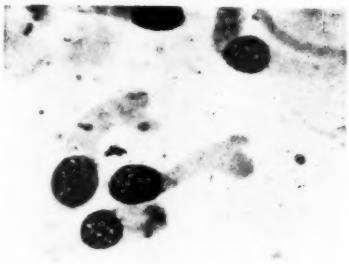

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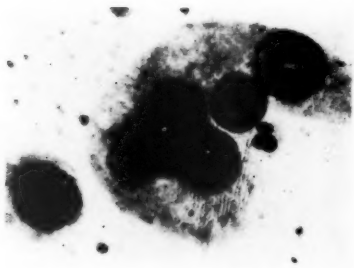

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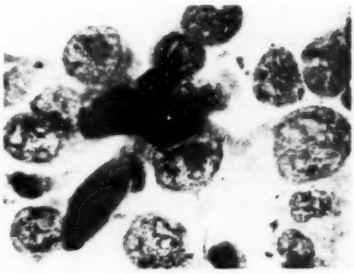

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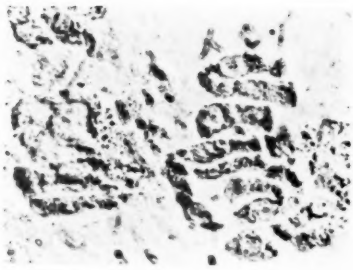

Fig. 1. Gastric abrasive balloon.

Fig. 2. Apex of the gastric abrasive balloon.

Fig. 3. Individually scattered gastric columnar cells. May-Grünwald-Giemsa's staining,—1000×.

Fig. 4. Tumour cells of gastric carcinoma with giant nuclei and giant nucleoli. May-Grünwald-Giemsa's staining,—1000×.

Fig. 5. A rosette of tumour cells of gastric carcinoma with polymorphism. May-Grünwald-Giemsa's staining,—1000×.

Fig. 6. Individually scattered gastric columnar cells. Phase-contrast-microscopic method,—1000×.

Fig. 7

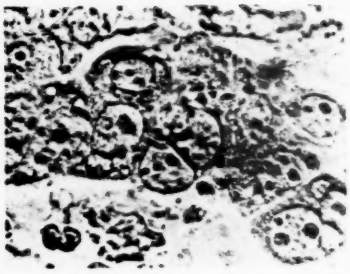

Fig. 8

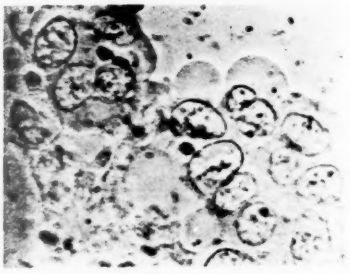

Fig. 9

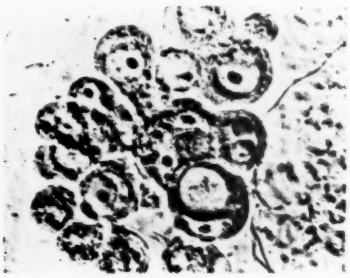

Fig. 10

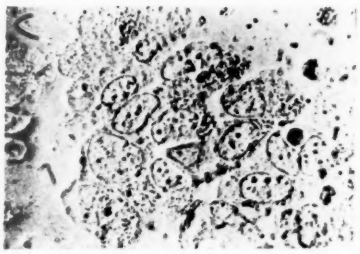

Fig. 11

Fig. 7. Tumour cells of gastric carcinoma with large nuclei and large nucleoli. Phase-contrast-microcspic method,—1000×.

Fig. 8. A combination of tumour cells of gastric carcinoma with photocytosis. Phase-contrast-microcopic method,—1000×.

Fig. 9. A rosette of tumour cells of gastric carcinoma with large nucleoli and secretory vacuolisation in cytoplasm. Phase-contrast-microscopic method, —1000×.

Fig. 10. A cluster of tumour cells of gastric carcinoma with polymmorphism. Phase-contrast-microscopic method,—1000×.

Fig. 11. A syncytium of tumour cells of gastric carcinoma with giant nuclei and giant nucleoli. Phase-contrast-microscopic method,—1000×.

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A Comparative Study of Results of Radiological and Cytological Tests

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It goes without saying that x-ray examination is of highest value in the diagnosis of gastric cancer in general. There are a number of complementary diagnostic methods next in importance to it and particularly, cytological diagnosis of gastric cancer has recently come to be evaluated very highly.[1-26] The present authors have already published several reports on cytological diagnosis of gastric cancer by the gastric abrasive balloon method and described its superiority.[27-29] In this paper, the results of cytological diagnosis is mentioned in comparison with the results of roentgenographical diagnosis.

Experimental

Material and Method

The majority of the subjects in this series were selected from in-patients and a smaller number of them from out-patients of this clinic with confirmed or suspected gastric cancer. A small number of patients with gastric ulcer and gastritis were studied as controls.

For collection techniques of cytological diagnosis, the gastric abrasive balloon was most frequently employed, but on very few occasions, gastric lavage with physiological saline or Ringer's solution was employed.

For curtailing the time required for collecting cells and for facilitating the exfoliation of cells from foci giving a suspicion of malignancy, x-ray findings were for reference in cytological diagnosis, but for judgment of the findings, the cytological and roentgenographical findings were examined independently and separately.

Classification of Cytological Findings

Cytological findings are classified in this clinic on the basis of the

following schema after Papanicolaou:

Class I—Absence of atypical or abnormal cells.

Class II—Atypical cells present but no evidence of malignancy.

Class III—Cells present giving a suspicion of malignancy.

Class IV—Fairly conclusive evidence of a malignant neoplasm.

Class V—Conclusive evidence of a malignant neoplasm.

The findings of Class I and II are judged as negative, Class III as suspicious and Class IV and V as positive for cancer.

Classification of Roentgenographical Findings

X-ray pictures taken prior to the application of cytological tests alone were used in this connection. For facilitating comparison with the results of cytological diagnosis, roentgenographical findings are classified similarly into the following 5 classes:

Class I—Absence of atypical or abnormal findings.

Class II—Abnormal findings present but no evidence of malignancy (including the findings of gastritis, gastric ulcer and so on).

Class III—Findings present upon whom a malignant neoplasm or a malignant alteration of benign lesion is not completely excluded, or the presence of such malignant formation or alteration is rather suspicious.

Class IV—Fairly conclusive findings of a malignant neoplasm.

Class V—Conclusive findings of a malignant neoplasm, showing the typical findings belong to any of the 4 types of the roentgenographical classification of gastric cancer established at this clinic.*

The findings of Class I and II are judged as negative, Class III as suspicious and Class IV and V as positive for cancer.

* The roentgenographical classification of gastric cancer was succinctly furnished by Kurokawa. These are as follows:

Type I—Polypoid type of carcinoma protruding into the gastric lumen. A filling defect resembling the impression of a loophole or a window in the barium column arises from the mass. The roentgenographical type, therefore, is described as the "loophole form" or the "window form."

Type II—Malignant ulcerative type of carcinoma. The roentgenographical sign is characterized with an ulcer niche on top of a filling defect from a heaped-up mass of neoplastic tissue protruding into the gastric lumen and seen face-on as a halo about the niche. The roentgenographical type is described as the "swimming tire form", because the filling defect produced by this type of carcinoma resembles the impression of a swimming tire on the barium pool.

Type III—Infiltrating scirrhous type of carcinoma. This spreads diffusely through the gastric walls both superficially and deeply and leads

to extreme narrowing of the gastric lumen surrounded by the thick and rigid walls of the stomach. The roentgenographical type is described as the " iron pipe form " because of the thick and rigid walls resembling the impression of an iron pipe.

Type IV—Mixed type of carcinoma. This represents a mixture of above three types of carcinoma and therefore, the roentgenographical appearance shows a combined of the above three forms, that is a " mixed form ". It also often shows the " honey comb form " resembling the impression of a honey comb.

Results

I. Over-all results of cytological diagnosis

Of the 831 patients studied by the end of November, 1958, gastric cancer was proved at operation or autopsy or was diagnosed as positive for cancer on the basis of definite clinical findings in 303. The remaining 528 were patients with other benign diagnosis.

The over-all results shown in Table I indicate that in the cancer group 90.4 per cent were correctly reported as cytologically positive or

TABLE I

Over-all Results of Cytological Diagnosis in 831 Cases

	Total	Cytology				
		Negative		Suspicious	Positive	
		I	II	III	IV	V
Cancer	303	8	21	16	58	200
		9.6%		5.3%	85.1%	
				90.4%		
No cancer	528	155	358	5	6	4
		97.2%		0.9%	1.9%	
				2.8%		
Total	831	163	379	21	64	204

suspicious, 9.6 per cent failed to exhibite cells suspicious or positive for cancer. In the non-cancerous group 97.2 per cent were correctly interpreted as negative but 2.8 per cent were incorrectly read as suspicious or positive for cancer.

II. Comparison of results: cytological diagnosis vs. radiological diagnosis

A comparative study of the results of cytological and radiological diagnoses was conducted in 228 of the 303 cancerous cases and in 461 of

TABLE II

Comparison of Results: X-Ray Diagnosis vs. Cytological Diagnosis in the 228 Cancerous Cases and the 461 Non-cancerous Cases

		X-ray diagnosis			Cytological diagnosis			Combined application*		
		Negat.	Susp.	Posit.	Negat.	Susp.	Posit.	Negat.	Susp.	Posit.
Cancer 228 cases	Number of cases	2	32	194	24	9	195	2	10	216
	%	0.9%	14.0%	85.1%	10.5%	4.0%	85.5%	0.9%	4.4%	94.7%
			99.1%			89.5%			99.1%	
No cancer 461 cases	Number of cases	253	190	18	447	5	9	449	7	5
	%	54.9%	41.2%	3.9%	97.0%	1.0%	2.0%	97.4%	1.6%	1.1%
			45.1%			3.0%			2.6%	

* Diagnosis by combined application of x-ray and cytological examinations.
Negat.—Negative Susp.—Suspicious Posit.—Positive

the 528 non-cancerous cases, or in 689 cases in total.

1. Comparison of over-all results (Table II)

In the 228 cancerous cases, the over-all results shown in Table II indicated that the accuracy of positive or suspicious diagnosis by radiological examination (99.1 per cent) is higher than that by cytological examination, but the accuracy of positive diagnosis (excluding suspicious diagnoses) is the approximate equal value of 85 per cent with both methods. The rate of suspicious diagnoses was much higher in radiological examination (14.0 per cent) than in cytological examination (4.0 per cent). When both methods of study were used in combination, the accuracy of positive diagnosis (94.7 per cent) rose higher than by single application of any of these diagnostic methods.

In the 461 non-cancerous cases, the accuracy of cytological diagnosis (97.0 per cent) was much higher than that of radiological diagnosis (54.9 per cent). A much higher percentage (45.1 per cent) of suspicious (41.2 per cent) or positive reports (3.9 per cent) was obtained by radiological examination, while by cytological examination a much smaller percentage (3.0 per cent) of such incorrect reports was obtained. By a combined application of both methods the ccuracy of acorrect diagnosis could be raised to 97.4 per cent.

2. Comparison of results by location of lesions (Tables III and IV)

In the neoplasms of the cardia-fornix the cytological and radiological tests, both achieved success in 100 per cent (including suspicious diagnosis) and in those of the corpus the accuracy of positive or suspicious diagnosis was much better with radiological examination (98.1 per cent) than with

TABLE III

Comparison of Results by Location of Neoplasms: X-Ray Diagnosis vs. Cytological Diagnosis in 59 Cases with the Neoplasm of the Cardia-Fornix, 108 Cases with the Neoplasm of the Corpus and 61 Cases with the Neoplasm of the Antrum-Pylorus

		X-ray diagnosis			Cytological diagnosis			Combined application*		
		Negat.	Susp.	Posit.	Negat.	Susp.	Posit.	Negat.	Susp.	Posit.
Cardia-fornix 59 cases	Number of cases	0	11	48	0	0	59	0	0	59
	%	0%	18.6%	81.4%	0%	0%	100.0%	0%	0%	100.0%
			100.0%			100.0%			100.0%	
Corpus 108 cases	Number of cases	2	15	91	12	5	91	2	8	98
	%	1.9%	13.9%	84.3%	11.1%	4.6%	84.3%	1.9%	7.4%	90.7%
			98.1%			88.9%			98.1%	
Antrum-pylorus 61 cases	Number of cases	0	6	55	12	4	45	0	2	59
	%	0%	9.8%	90.2%	19.7%	6.6%	73.8%	0%	3.3%	96.7%
			100.0%			80.3%			100.0%	

* Diagnosis by a combined application of x-ray and cytological examinations.
Neg.—Negative Susp.—Suspicious Posit.—Positive

TABLE IV

Comparison of Results by Location of Lesions: X-Ray Diagnosis vs. Cytological Diagnosis in the 113 Cases of the Cardia-Fornix, the 242 Cases of the Corpus and the 106 Cases of the Antrum-Pylorus, in Total 461 Cases without Cancer

		X-ray diagnosis			Cytological diagnosis			Combined application*		
		Negat.	Susp.	Posit.	Negat.	Susp.	Posit.	Negat.	Susp.	Posit.
Cardia-fornix 113 cases	Number of cases	52	57	4	108	1	4	108	4	1
	%	46.0%	50.4%	3.6%	95.6%	0.9%	3.6%	95.6%	3.6%	0.9%
			54.0%			4.4%			4.4%	
Corpus 242 cases	Number of cases	144	90	8	235	3	4	236	3	3
	%	59.5%	36.7%	3.8%	97.1%	1.2%	1.7%	97.6%	1.2%	1.2%
			40.5%			2.9%			2.4%	
Antrum-pylorus 106 cases	Number of cases	57	43	6	105	0	1	105	0	1
	%	55.4%	40.6%	3.7%	99.1%	0%	0.9%	99.1%	0%	0.9%
			44.2%			0.9%			0.9%	

* Diagnosis by a combined application of x-ray and cytological examinations.
Negat.—Negative Susp.—Suspicious Posit.—Positive

cytological examination (88.9 per cent). When neoplasms were located in the antrum-pylorus, radiological examination bore 100 per cent successful reports, but the accuracy of cytological diagnosis was rather low (80.3 per cent). The accuracy of positive diagnosis (excluding suspicious diagnosis) was higher with cytological diagnosis (100 per cent) than with radiological diagnosis (81.4 per cent) in the neoplasms of the cardia-fornix, and in those of the corpus was equal with both radiological and cytological diagnoses (84.3 per cent). While in the neoplasms of the antrum-pylorus the accuracy of positive diagnosis was higher with radiological examination (90.2 per cent) than with cytological examination (73.8 per cent) and the high accuracy of positive diagnosis (96.7 per cent) was obtained with a combined application of both methods.

In the next place, the neoplasms of 22 cases could not be confirmed by radiological examination but cytological examination. One of them with neoplasm of the cardia-fornix showed negative findings upon radiological examination, while the remaining 11 cases with neoplasms of the cardia-fornix, 8 cases of the corpus and 2 cases of the antrum-pylorus showed suspicious findings in their roentgenograms, but positive findings upon cytological tests. Such neoplasms of discrepant results were more frequent among the neoplasms of the cardia-fornix more difficult to detect roentgenographically.

In the non-cancerous cases, the accuracy of radiological diagnosis was a half of cases wherever the suspected lesions of the stomach are located, while the accuracy of cytological diagnosis was more than 95 per cent of the cases.

3. Comparison of results in the cases with intramucosal carcinoma

Six intramucosal carcinomas were histologically proved in the stomach which had undergone a gastric resectomy. All of these carcinomas showed positive findings radiologically, while five among them were correctly reported as cytologically positive, but another failed to exhibite cells either suspicious or positive because of the small tumor scarcely cropping out on the surface of the mucous membrane.

4. The relation between roentgenographical types of neoplasms and results of cytological diagnosis (Table V)

Roentgenographical types of gastric cancer were devided into four, according to the roentgenographical classification by Kurokawa, as mentioned already, and the results were studied in relation with results of cytological diagnosis.

The accuracy of cytological diagnosis was 91 per cent in the neoplasms of Types I, III and IV and 84.3 per cent in those of Type II. In the neoplasms of the cardia-fornix, the accuracy was rather low in Type III than in Type I, II, and IV, in the neoplasms of the corpus, the highest

TABLE V

The Relation between Roentgenographical Types of Neoplasms (by Kurokawa's Classification) and Cytological Test Results

Roentgenographical classification of gastric cancer by Kurokawa	Cardia-fornix			Corpus			Antrum-pylorus			Total		
	Num.	Pos.	Accur.	Num.	Pos.	Accur.	Num.	Pos.	Accur.	Num.	Pos.	Accur.
Type I (loophole form)	6	6	100.0%	11	11	100.0%	6	4	66.7%	23	21	91.3%
Type II (swimming form)	32	32	100.0%	59	49	83.1%	36	26	72.2%	127	107	84.3%
Type III (iron pipe form)	19	18	94.7%	26	25	96.2%	15	12	80.0%	60	55	91.7%
Type IV (mixed form)	12	12	100.0%	47	42	89.4%	30	27	90.0%	89	81	91.0%

Num.—Number of cases.
Pos.—Number of cases with positive report.
Accur.—Accuracy of positive diagnosis.

in Type I and lower in Types III, IV and II in the order named, and in those of the antrum-pylorus, the highest in Type IV followed by Types III, II and I in descending order.

5. Comparison of results in the cases with ulcerating lesions of the stomach (Table VI)

There were the 88 cases with malignant ulcer, that is, the 12 cases with cancer-ulcer and the 76 cases with cancerous ulcer, and the 168 cases with benign gastric ulcer.

The accuracy of positive diagnosis in the 88 malignant ulcers of the stomach was 84.1 per cent with radiological examination, 81.8 per cent with cytological examination and 91.0 per cent with combined application of both methods respectively.

In the 12 ulcer-cancers of the malignant ulcers, the accuracy of positive or suspicious diagnosis was 83.3 per cent with radiological examination and 66.7 per cent with cytological examination, but if the suspicious diagnoses are excluded, the accuracy falls to the low level of 50.0 per cent with radiological and 41.7 per cent with cytological examinations, not rising beyond 66.7 per cent even with combined application of both methods. If the suspicious diagnoses are included, the accuracy was rather high (91.7 per cent) with combined application of both methods.

In the 76 cancerous ulcer, the accuracy of positive diagnosis was rather high with any of these diagnostic methods, particularly with combined application of both methods (96.1 per cent).

In the 168 benign gastric ulcers, the rate of suspicious or false-positive diagnoses was 40.5 per cent with radiological and 4.2 per cent with cyto-

TABLE VI

Comparison of Results in Cases with Ulcerating Lesions: X-Ray Diagnosis vs. Cytological Diagnosis in the 88 Cases with Malignant Ulcer and the 168 Cases with Benign Ulcer

		Number of cases	X-ray diagnosis			Cytological diagnosis			Combined application*		
			Negat.	Susp.	Posit.	Negat.	Susp.	Posit.	Negat.	Susp.	Posit.
Malignant ulcer	Total	88	3 3.4%	11 12.5%	74 84.1%	13 14.8%	3 3.4%	72 81.8%	2 2.3%	5 5.7%	81 91.0%
				96.6%			85.2%			96.7%	
	Ulcer-cancer	12	2 16.7%	4 33.3%	6 50.0%	4 33.3%	3 25.0%	5 41.7%	1 8.3%	3 25.0%	8 66.7%
				83.3%			66.7%			91.7%	
	Cancerous ulcer	76	1 1.3%	7 9.2%	68 89.5%	9 11.8%	0 0%	67 88.2%	1 1.3%	2 2.6%	73 96.1%
				98.7%			88.2%			98.7%	
Benign ulcer	Total	168	100 59.5%	62 36.9%	6 3.6%	161 95.8%	2 1.2%	5 3.0%	162 96.4%	3 1.8%	3 1.8%
				40.5%			4.2%			3.6%	
	Callous ulcer	14	8 57.1%	5 35.7%	1 7.1%	12 85.7%	0 0%	2 14.3%	10 71.4%	1 7.1%	3 21.4%
				42.9%			14.3%			28.6%	
	Non-callous ulcer	154	92 59.7%	57 37.1%	5 3.2%	149 96.8%	2 1.3%	3 1.9%	152 98.7%	2 1.3%	0 0%
				40.3%			3.2%			1.3%	

* Diagnosis by a combined application of x-ray and cytological examinations.

Negat.—Negative Susp.—Suspicious Posit.—Positive

logical examinations respectively, but if the suspicious diagnoses are excluded, the rate of incorrect diagnoses is reduced to less than 4 per cent. Thus, suspicious diagnosis being rather frequent with radiological examination and being rare with cytological examination respectively, a combined application of these two methods will surely enhance the accuracy of diagnosis to a extremely high level.

When the benign gastric ulcers are devided into the callous and non-callous ulcers, the rate of incorrect diagnoses is higher by many folds in the callous ulcers than in the non-callous ulcers with any of the diagnostic methods. Particularly, the high rate of incorrect diagnoses (14.3 per cent) upon cytological tests in the callous ulcers should be noted.

6. Comparison of results in the cases with benign polyp or polyposis, malignant polyp, gastritis and other benign diseases (Table VII).

TABLE VII

Comparison of Results: X-Ray Diagnosis vs. Cytological Diagnosis in the 17 Cases with Benign Polyp or Polyposis, the 4 Cases with Malignant Polyp, the 217 Cases with Gastritis and the 59 Cases with other Benign Diseases

	Number of cases	X-ray diagnosis			Cytological diagnosis			Combined application*		
		Negat.	Susp.	Posit.	Negat.	Susp.	Posit.	Negat.	Susp.	Posit.
Benign polyp and polyposis	17	9 52.9%	6 35.3% 47.1%	2 11.8%	15 88.2%	1 5.9% 11.8%	1 5.9%	15 88.2%	1 5.9% 11.8%	1 5.9%
Malignant polyp	4	0 0%	1 25.0% 100.0%	3 75.0%	1 25.0%	0 0% 75.0%	3 75.0%	0 0%	0 0% 100.0%	4 100.0%
Gastritis	217	104 47.9%	106 48.9% 52.1%	7 3.2%	213 98.2%	1 0.5% 1.8%	3 1.4%	214 98.6%	2 0.9% 1.4%	1 0.5%
Others	59	40 67.8%	16 27.1% 32.2%	3 5.1%	58 98.3%	1 1.7% 1.7%	0 0%	58 98.3%	1 1.7% 1.7%	0 0%

* Diagnosis by a combined application of x-ray and cytological examinations.
Negat.—Negative Susp.—Suspicious Posit.—Positive

In the 17 benign polyp or polyposis of the stomach, the results showed similar tendencies as observed the benign gastric ulcers.

In the 4 polyps with malignant alteration into carcinoma, the accuracy of diagnosis was 75 per cent with either radiological or cytological examination, but upon combined application of both methods, the accuracy attained 100 per cent of the cases.

In the cases with gastritis or other benign diseases of the stomach, the tendencies of the results was the same as in the cases with benign gastric ulcer.

CONCLUSIONS

Experience with gastric cytologic study in 831 patients has been reviewed, and particularly, comparisons have been made between the accuracy of radiological and cytological diagnoses in 689 of these patients.

It goes without saying that radiological examination is indispensable in the diagnosis of the pathologic conditions of the stomach, especially, gastric cancer and the accuracy of correct diagnosis obtained thereby is high.

Radiological and cytological diagnoses are almost equally reliable in reporting malignant disease of the stomach in this series. The overlapping, combined accuracy of these two methods, however, appreciably increases the accuracy of this diagnosis in the doubtful or indeterminate group of pathologic conditions in the stomach. Cytological study should therefore be used in conjunction with and not in competition with radiological study of these difficult cases. Although disclosure of tumor cells is almost always confirmatory of gastric malignancy and absence of tumor cells suggests a benign lesions, failure to identify tumor cells does not rule out malignancy. Besides, cytological examination does not furnish data for diagnosing gastric ulcer, gastric polyp, gastritis and other benign gastric lesions. In addition, gastric cytologic analysis is powerless in giving informations on the location, the extent or size of neoplasms or the indication of surgical operation. These informations must be sought by radiological examination which also gives a clew to more appropriate application of cytological examination.

In summary, the adjunctive or complementary rather than competitive approch in the application of cytology and radiography is emphasized.

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Fundamental Studies on Cytological Diagnosis of Gastric Cancer

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The significance of cytological diagnosis of gastric cancer has recently come to be appreciated very highly and many reports have appeared on the subject.[1-18] In the experience with gastric cytologic study by the abrasive balloon, the present authors[19-24] have also confirmed that gastric desquamative cytology is a very helpful method as one of the complementary rather than competitive test to gastric radiology in the diagnosis of gastric cancer. There are, however, several pittsfalls in the application of the principles of cytological analysis. In view of growing interest in this method of study, it is recommended that no one attempts it without first securing the aid of an expert cytologist, since, in addition to the skill and technique obtaining gastric specimens for study, the results will depend upon the ability of the cytologist to interpret them. In this meaning, the present authors were prompted to undertake some fundamental study on the criteria of malignancy applied to desquamated cells and morphologic changes of cancer cells induced by various types of mounting media.

Experimental

Methods, Materials and Results

I. Cytologic criteria of malignancy

Smear preparations were directly made from cancerous foci and noncancerous foci in the stomach which had undergone a gastric resectomy and were examined under the phase-contrast microscope or after the May-Grünwald-Giemsa's staining.

Normal epithelium can be hardly smeared in the single cells scattered

throughout the smear but cell clusters or small tissue fragments from the surface of the mucous membrane of the stomach even by way of the scraping preparation. Tumor cells are, however, so liable to desquamate that many tumor cells can be freely smeared. In smears a glimps of the tissue pattern is afforded by cell clusters or small tissue fragments which are frequently found intermixed with the single cells.

The criteria of malignancy in desquamative cytology are based on changes in structure of individual cells as well as on their altered interrelationships. However, interest is centered primarily on the morphologic characteristics of the individual cells.

A. Structural modifications of cells and their nuclei

To establish the criteria of malignancy based on structural modifications of cells and their nuclei, the fully detached cells which are seen in toto were selected from among the cells in smear and analysed cytologically, because of them giving the more complete picture of these individual structure.

As shown in Table I, cellular and nuclear enlargement, disproportionate nuclear enlargement, aberrance in the nuclear form, marked thickning of the nuclear membrane, hyperchromasia, enlarged nucleoli and increase in number of nucleoli were most frequently observed as morphologic aberrations of tumor cells. A single criterion by itself, however, such as cellular and nuclear enlargement, marked thickning of the nuclear membrane, aberrance in the nuclear form, naked nucleus, enlarged nucleoli, increase in number of the nucleoli, etc., should not be stressed in the cancer cells of the stomach, as it may be seen in chronic inflammation and other non-malignant pathologic conditions of the stomach. Despite these structural abberations from the normal type, the non-malignant nature of the cells can be recognized by the fact that they have certain criteria of normalcy, such as a normal chromatin pattern or a normal nuclear-cytoplasmic ratio.

B. Changes in the interrelationships of cells and their nuclei

To establish the criteria of malignancy based on changes in the interrelationships of cells and their nuclei, cell clusters and small tissue fragments in smear were selectively analysed cytologically. These results are summarized as shown in Table II. The cancer cells and their nuclei were found densely grouped or croweded one above another and no regularity could be at al observed in their orientation and arrangement. They showed marked variation in their size (anisocytosis and anisokaryosis), their form (poikilocytosis and poikilokaryosis), and frequently irregularity of pattern of their staining reaction and degeneration. These changes in the interrelationships of cells and their nuclei may be also observed singly in apparently normal cell clusters or small tissue fragments.

TABLE I

Criteria Based on Structural Modifications of Cells and their Nuclei

Criteria of malignancy	Tumor cells		Non-malignant cells	
	P.C.M.	M.G.G.	P.C.M.	M.G.G.*
(1) Enlargement of cells and nuclei beyond their normal range	59.8%	72.0%	6.2%	22.6%
(2) Aberrance in the form of the cell	6.6%	1.3%	0.8%	0.2%
(3) Disproportionate enlargement of the nucleus, altering appreciably the normal nuclear-cytoplasmic ratio	56.1%	50.3%	(49.8%)**	8.0%
(Enlargement of the cells with non-predominant nucleus)	(16.0%)	(18.5%)	—	—
(4) Nucleus				
a) Aberrance in the form of the nucleus	20.9%	47.2%	5.4%	8.0%
b) Multinucleation	6.3%	3.7%	0.2%	0.4%
c) Naked nucleus	11.2%	12.7%	10.0%	9.4%
d) Marked thickning of the nuclear membrane	44.5%	50.3%	7.8%	13.2%
e) Increase in the chromatin content, causing hyperchromasia	—	20.6%	—	4.8%
f) Decrease in the chromatin content, causing hypochromasia	—	2.9%	—	4.6%
(5) Nucleolus				
a) Englarged nucleoli beyond their normal range	48.1%	37.0%	6.0%	7.2%
b) Increase in number of the nucleoli beyond their normal range	25.1%	39.3%	6.6%	8.4%
c) Aberrance in the form of the nucleolus	16.3%	24.5%	1.0%	2.8%
d) Extremely eccentric localized nucleoli in the nucleus	17.1%	—	1.6%	—
(6) Changes of the cytoplasm reflected in the staining reaction	—	8.6%	—	5.8%
(7) Marked vacuolation of the cytoplasm	2.3%	12.8%	1.1%	6.2%

* P.C.M.—Phase-contrast microscopic method.
M.G.G.—May-Grünwald-Giemsa's method.

** Percentage of parenthesis represents the frequency of enlargement of nuclei in the cells in surface view.

In surveying the above criteria it becomes apparent that no single criterion is sufficient to establish conclusively the presence of malignancy. Only the fulfillment of a number of criteria provide sufficient evidence for malignancy.

C. Variability of the maximum diameter of the nuclei

As estimated in living condition of the cells under a phase-contrast microscope, the maximum diameter of the nuclei of non-malignant epi-

TABLE II

Criteria Based on Changes in the Interrelationships of Cells and their Nuclei

	A cluster or tissue fragment of non-malignant cells	A cluster or tissue fragment of malignant cells
Pattern of arrangement	Relatively regular cell arrangement with individual cells separated by distinct borders and their nuclei separated at a certain distance each other	Irregular cell arrangement with lack of uniformity in the orientation and dense grouping and crowding of cells and their nuclei, or with lack of distinct cell boundaries
Size of nuclei and cells	Uniform or slight variation	Marked variation: Anisokaryosis and anisocytosis
Form of nuclei and cells	Uniform or slight variation	Marked variation: Poikilokaryosis and poikilocytosis
Pattern of the staining reaction	Uniform pattern	Irregularity of pattern
Degenerative or necrotic changes	Slight	Frequently marked

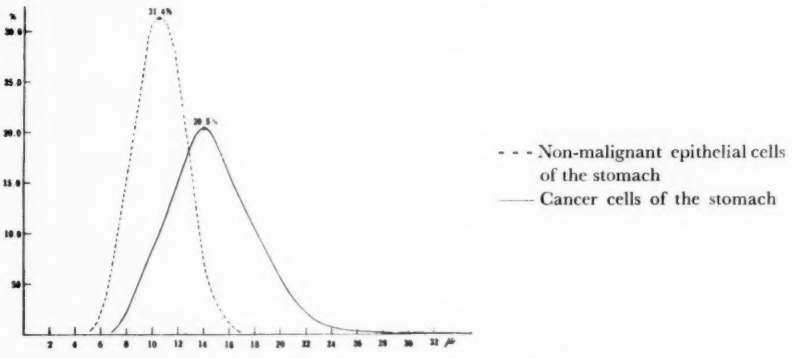

Fig. 1. Variability of the maximum diameter of the nuclei in gastric cancer cells and non-malignant gastric epithelial cells. (May-Grünwald-Giemsa's staining method).

thelial cells ranged between 5 and 17 μ averaging about 10.5 μ, while that of cancer cells stood between 7 and 30 μ, the majority measuring about 14 μ (Fig. 1).

In smear stained according to the May-Grünwald-Giemsa's method, the non-malignant cell nuclei measured between 6 and 20 μ, those of about 10 μ being most frequent, while the nuclei of gastric cancer cells ranged between 7 and 32 μ in diameter, the most frequent size being around

14.5 μ (Fig. 2).

In surveying the above data it is understood confirmatively that the cancer cells show frequently the enlargement of nuclei beyond their normal range.

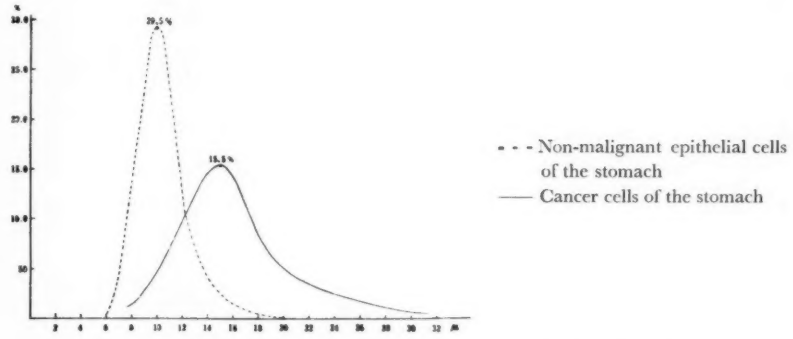

Fig. 2. Variability of the maximum diameter of the nuclei in gastric cancer cells and non-malignant epithelial cells. (Phase-contrast microscopic method).

II. Morphologic changes of cancer cells induced by various types of mounting media

The cancer cells were obtained directly from the cancerous foci of the stomach which had undergone a gastric resectomy by way of the scraping preparation and were suspended in various types of mounting media under a cover slip, and their morphologic changes were observed in the course of time with phase-contrast microscopy.

a) 5% glucose solution

In the medium of 5% glucose solution, the cancer cells were well preserved in their structure even for 5 or 8 hours after cells were suspended, although a slight degree of cellular damage occurs in the course of time (Figs. 3 and 4).

b) Physiological saline

In earliest time, physiological saline (0.9%) induced no perceptable changes of cancer cells in structure. However, the swelling of cytoplasm and nucleus, the indistinctness of their structure and membranes, and the formation of blebs or bubbles in the cytoplasm, etc., were observed in the course of time (Figs. 5 and 6). The degree of such degenerative changes was medium even at 8 hours after cells were suspended in saline medium.

c) Human blood plasma

In human blood plasma, the cancer cells were preserved most intactly

for 5 or 8 hours (Figs. 7 and 8).

d) Gastric juice

In the gastric juice of achlorhydria which contains 0 unit of free acidity and 30 units of total acidity, the cancer cells showed the degenerative changes in their structure, for example, the formation of vacuoles and blebs or bubbles in the cytoplasm, the indistinctness of cytoplasmic and nuclear boundaries, etc. Such degenerations remained in the slight degree for 30 minutes to 3 hours after cells were suspended in the above medium and aggrevated to the medium degree in the course of 5 or 8 hours (Figs. 9 and 10).

In the normacid gastric juice which contains 36 units of free acidity and 42 units of total acidity, the cancer cells showed the severe degree of the above degenerations in 30 minutes already. In addition, the pyknotic changes in the cytoplasm and the nucleus, the naked nuclei, etc., were appeared in the course of time and lastly the degenerations became so marked that the tumor cells could not be identified as such (Fig. 11). In the hyperacid gastric juice, such degenerations were observed in the more severe degree, but their pattern was the same.

e) Pepsin solution

A slight degeneration was observed in the cancer cells kept for 3 hours in the medium of 0.2% pepsin solution and only a slight aggravation of the degeneration could be recognized in the course of 5 to 8 hours (Figs. 12, 13 and 14).

f) Hydrochloric acid solution

The medium of 0.1 N hydrochloric acid solution induced only a slight degeneration of cancer cells even in the course of 5 hours (Figs. 15 and 16). In 1 N hydrochloric acid solution, however, a very marked degeneration could be affirmed in 30 minutes already and their degree became more severe in the course of time. The pattern and degree of degeneration were similar to those induced by the normacid or hyperacid gastric juice (Fig. 17).

g) Pepsin-hydrochloric acid solution

In the pepsin-hydrochloric acid solution of the equal parts of 0.2% pepsin and 0.1 N hydrochloric acid solutions, the very marked degenerations were observed already in the earliest time and their pattern and degree were similar to those induced by the hyperacid gastric juice or 1 N hydrochloric acid solution. That is to say, such degenerations were more marked than those induced by any of both 0.2% pepsin and 0.1 N hydrochloric acid solutions (Fig. 18).

In surveying the above result summarized in Table III, it is clearly indicated that 5% glucose solution and human blood plasma are most commendable as medium for keeping the specimens for cytological diagno-

TABLE III

Degree of Morphologic Changes of Cancer Cells Induced by Various Types of Mounting Media

Mounting media		Degree of morphologic change			
		30 min.	3 hrs.	5 hrs.	8 hrs.
5% glucose solution		Slight	Slight	Slight	Slight
Physiological saline (0.9%)		Medium	Medium	Medium	Medium
Blood plasma		Slight	Slight	Slight	Slight
Gastric juices	Free acidity 36 Total acidity 42	Severe	More severe		——
	Free acidity 0 Total acidity 30	Slight	Slight	Medium	Medium
0.2% pepsin solution		Slight	Slight	Slightly medium	
0.1 N hydrochloric acid solution		Slight	Slightly medium		
1 N hydrochloric acid solution		Severe	More severe		
The medium of the equal parts of 0.2% pepsin and 0.1 N hydrochloric acid solutions		Severe	More severe		

sis of gastric cancer, as well as that degeneration occurs promptly in the hyperacid gastric juice, and particularly the presence of both free hydrochloric acid and pepsin seems to play an important parts in inducing cellular damage.

SUMMARY

With the aim of furnishing fundamental data for cytological diagnosis of gastric cancer, two experiments were made. In one, direct smear preparations were made from the cancerous and non-cancerous foci of the stomach which had undergone a gastric resectomy and were examined to establish the criteria of malignancy based on cytological features. It became thereby apparent that no single criterion is sufficient to establish conclusively the presence of malignancy, but the fulfillment of a number of criteria provide sufficient evidence for malignancy. In the other experiment, the action of various types of mounting media on cancer cells was studied with phase-contrast microscopy. It was thereby clearly indicated that 5% glucose solution and human blood plasma are commendable as medium for keeping the specimens for cytological diagnosis of gastric cancer, as well as that the presence of both free hydrochloric acid and pepsin seems to play an important parts in inducing cellular damage.

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Fig. 3

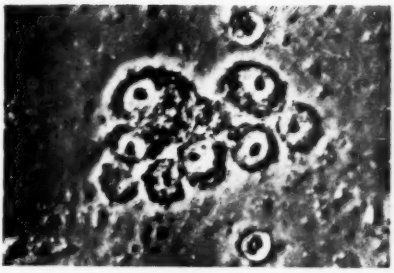

Fig. 4

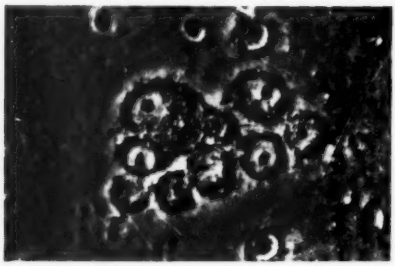

Fig. 5

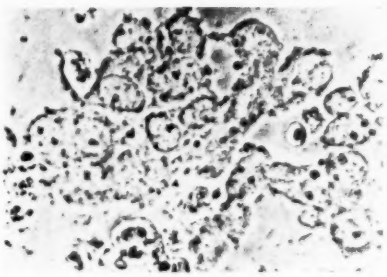

Fig. 6

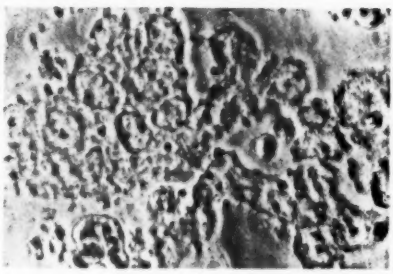

Fig. 7

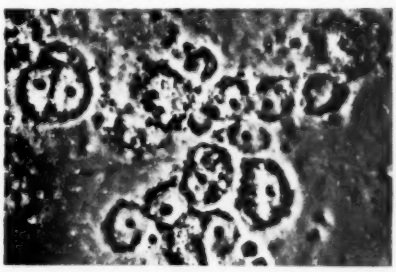

Fig. 8

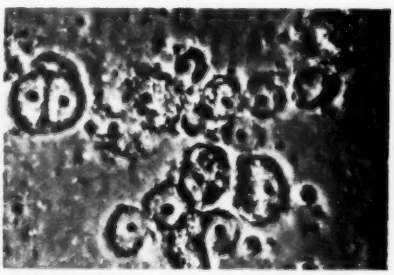

Fig. 3. Cancer cells kept for 3 hours in the medium of 5% glucose solution, showing the slight degree of morphologic changes.

Fig. 4. Cancer cells kept for 8 hours in the medium of 5% glucose solution, showing the slight degree of morphologic changes.

Fig. 5. Cancer cells kept for 1 hour in the medium of physiological saline, showing the medium degree of morphologic changes.

Fig. 6. Cancer cells kept for 5 hours in the medium of physiological saline, showing the medium degree of morphologic changes.

Fig. 7. Cancer cells kept for 3 hours in the medium of human blood plasma, showing the slight degree of morphologic changes.

Fig. 8. Cancer cells kept for 8 hours in the medium of human blood plasma, showing the slight degree of morphologic changes.

Fig. 9

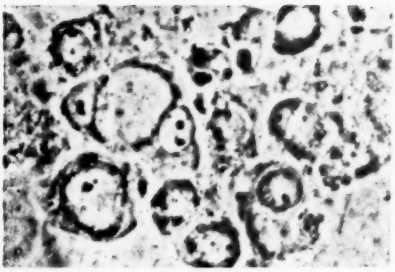

Fig. 10

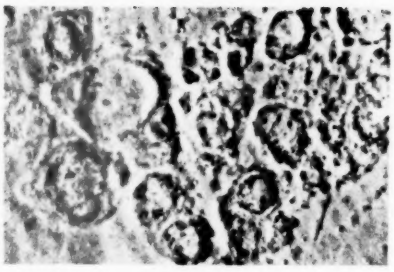

Fig. 11

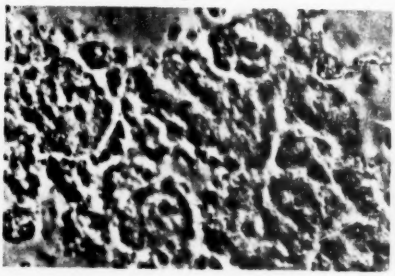

Fig. 12

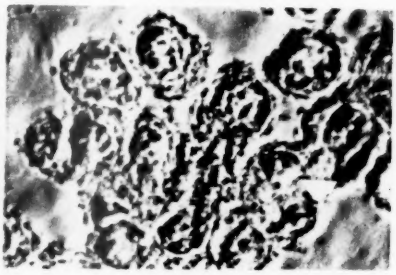

Fig. 13

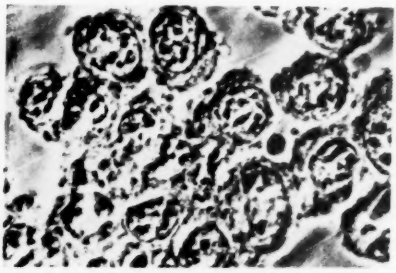

Fig. 14

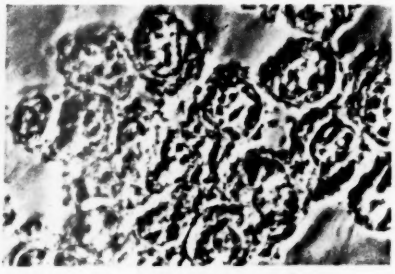

Fig. 9. Cancer cells kept for 1 hour in the medium of gastric juice (free acidity 0, total acidity 30), showing the slight degree of morphologic changes.

Fig. 10. Cancer cells kept for 5 hours in the medium of gastric juice (free acidity 0, total acidity 30), showing the medium degree of morphologic changes.

Fig. 11. Cancer cells kept for 30 minutes in the medium of gastric juice (free acidity 36, total acidity 42), showing the severe degree of morphologic changes.

Fig. 12. Cancer cells kept for 30 minutes in the medium of 0.2% pepsin solution, showing the slight degree of morphologic changes.

Fig. 13. Cancer cells kept for 3 hours in the medium of 0.2% pepsin solution, showing the slight degree of morphologic changes.

Fig. 14. Cancer cells kept for 5 hours in the medium of 0.2% pepsin solution, showing the slightly medium degree of morphologic changes.

Fig. 15

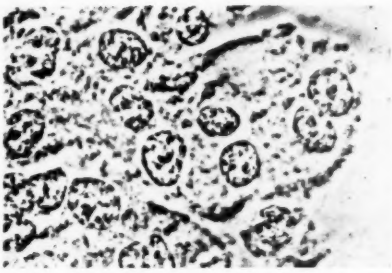

Fig. 16

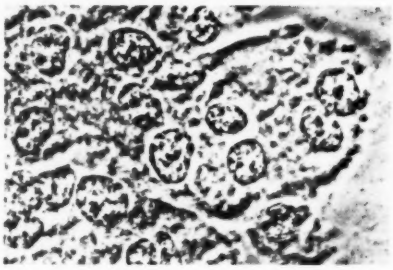

Fig. 17

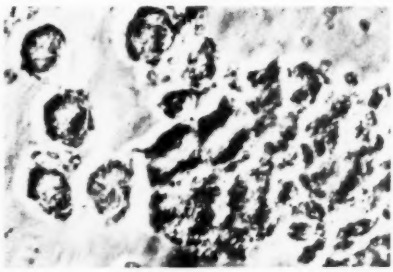

Fig. 18

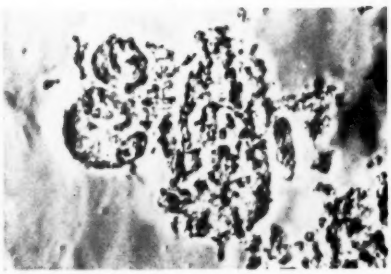

Fig. 15. Cancer cells kept for 30 minutes in the medium of 0.1 N hydrochloric acid solution, showing the slight degree of morphologic changes.

Fig. 16. Cancer cells kept for 5 hours in the medium of 0.1 N hydrochloric acid solution, showing the slightly medium degree of morphologic changes.

Fig. 17. Cancer cells kept for 30 minutes in the medium of 1 N hydrochloric acid solution, showing the severe degree of morphologic changes.

Fig. 18. Cancer cells kept for 30 minutes in the medium of the equal parts of 0.2% pepsin and 0.1 N hydrochloric acid solutions, showing the severe degree of morphologic changes.

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Effects of Gamma-Aminobutyric Acid and Gamma-Amino-Beta-Hydroxybutyric Acid on the Avoidance Conditioned Reflexes

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(Received for publication, September 5, 1959)

Introduction

According to the biochemical and neurophysiological investigations by many workers[1] there can be little doubt that γ-aminobutyric acid (GABA) or a closely related substance is concerned in some way with the transmission of nerve impulses in the central nervous system and that the mode of its action is inhibitory in nature, although the exact nature and site of its action are not yet clear. Hayashi[2] asserts that γ-amino-β-hydroxybutyric acid (GABOB) is more important than GABA as an inhibitory substance. If GABA or GABOB is closely related with the inhibitory processes within the central nervous system, it may be also connected with the internal inhibition of the conditioned reflex (CR).

It is, therefore, the purpose of the present study to investigate the effects of these substances on the avoidance CR in animals. In this field, it was reported by Suhara[3] that the positive CR of discrimination was always inhibited by oral administration of GABOB in the salivary CR in man.

Experimental

Method

Experiments were performed on adult dogs in which an avoidance CR was established by the so-called instrumental training method.

In this method the occurrence of a noxious stimulus was prevented automatically during occurrence of a CR. For training or testing the animals were gently restrained in a hammock which permitted freedom of movement of all four limbs. All experiments were performed in a sound-proof room. As the conditioned stimulus (CS) a 1000 c/s tone enduring about 1 sec. from an audio-frequency oscillator was used. Its

termination was overlapped with the unconditioned stimulus (US). The US, rhythmic electric shocks (20–40 V) from an electronic stimulator, was delivered to the right hind leg. One pole of the stimulating electrode was a needle inserted into the skin of the right hind leg, and the other a tinplate (30×20 cm) on which the leg was placed. One training session was 20 trials each except for discrimination training in which it was 30 trials each. The intervals between the separate trials varied from 30 sec. to 3 min. at random, in order to avoid temporal conditioning.

Discrimination training was attempted in some animals using alternatively a 1000 c/s tone as the positive stimulus and a 800 c/s tone as the negative one.

GABA or GABOB not only increased the latency of the CR, but also augmented its fluctuations. Therefore the standard deviation (SD) of latencies was utilized for characterizing the effects of the agents. When the latency became short and steady by sufficient reinforcement, the test agent was administered intravenously through a cutaneous vein of the left hind leg or intracisternally.

In the following experiments SDs of latencies obtained in successive 20 trials were measured immediately after, 5 hours after and 24 hours after the administration of the test agent.

Results

1. *Latency as an index of the central excitatory level*

As a preliminary experiment the latency of CR was investigated to see whether or not it might be used as an index of the central excitatory level.

About three weeks after the beginning of training (one session a day) the CR was established. When tested without reinforcement several days or longer after completion of training, animals were found to be thoroughly conditioned (see Fig. 1). Although the percentage frequency of CR (proportion of positive CRs to the number of trials in each session) remained 100% even without further reinforcement, it was noted that the latencies showed remarkable variations from about 0.5 to 1.5 sec. So further reinforcement with much shortened intervals between the CS and US was attempted to obtain a more rapid and steady response to the CS. After the further reinforcement the latencies became shorter and more regular, ranging from about 0.35 to 0.45 sec. (see Fig. 2).

Inhibition of CR may be caused by different kinds of experimental procedure, such as extinction, discrimination, delay, distraction and so on. Among these various procedures the experimental extinction and discrimination were attempted in the present experiments. In the experimental procedure of acute extinction in which the CS was repeatedly

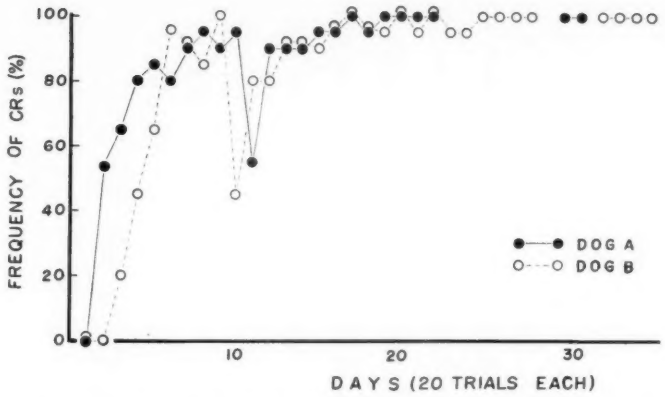

Fig. 1. Learning curves by instrumental avoidance training. Abrupt down-ward deflection of curves on the 10th day of training resulted from temporary suspension of reinforcement.

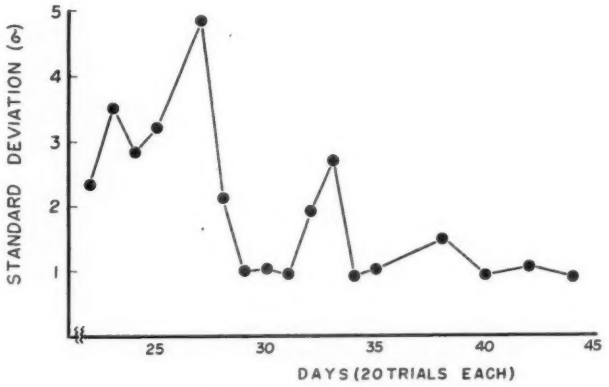

Fig. 2. Changes in standard deviations of latencies during the advanced course of conditioning. Experiments were started when percentage frequency became 100%.

presented without reinforcement, the latencies became irregular, showed a progressive prolongation and finally the percentage frequency of CR began to decrease (see Fig. 3). Whenever extinctive inhibition occurred, decrement of percentage frequency was preceded by the prolongation and irregularity of latencies.

Next, experiments of discrimination were carried out. In these experiments, even after training of about 50 days discrimination was not yet

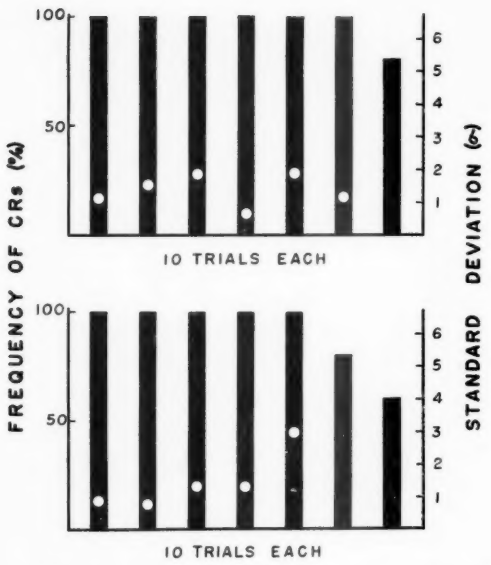

Fig. 3. Changes of percentage frequency and latency (standard deviation) in experimental extinction.

Filled bar: percentage frequency. Open circle: standard deviation of latencies.

well-established and apt to generalize (see Fig. 4). In the course of training it was found that in unsuccessful discrimination the latency of the positive CR was rather shorter and more regular, whereas it was unstable in successful discrimination (see lower diagram in Fig. 4). It seemed that conditions under which short and rigid latencies of CR were obtained were unfavorable for discrimination. The longer and variable latencies of CR in association with successful discrimination may be due to internal inhibition caused by negative stimuli.

From the above-mentioned experiments it may be considered that the latency and its fluctuation can serve as an index of the central excitatory level.

2. *Effects of GABA and GABOB administered intravenously upon conditioned responses*

No changes in the behavior and percentage frequency of CR were observed with doses of 25 to 100 mg./kg. The animals were as usual friendly or affectionate and showed interest in the environment. Irregularity or prolongation of latency, however, was noted immediately after and 5 hours after the injection. After 24 hours the latency was found to

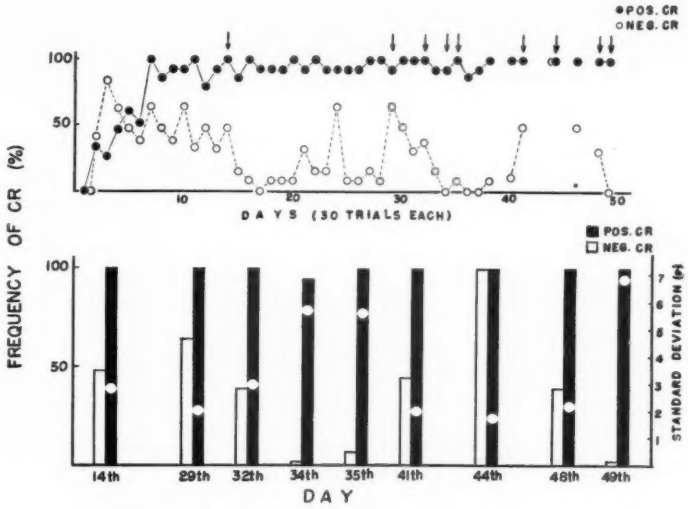

Fig. 4. Learning curves by discrimination training (upper graph). Filled circle: percentage frequency of CR to positive stimulus. Open circle: that of CR to negative stimulus. Difference between both percentage frequencies indicates degree of discrimination. Both percentage frequency of CR and SD of latency at points indicated by arrows in upper diagram are shown in lower diagram.

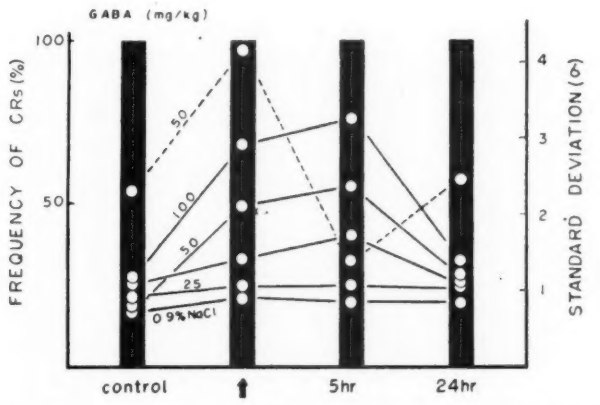

Fig. 5. Effects of intravenous administration of GABA in various doses. Filled bar: percentage frequency of CR. White circle: SD of latency. Arrow: immediately after injection of GABA. Exceptional case in which SD of latency was extraordinarily high is shown by broken curve.

have been restored to the initial level. The larger the doses of GABA, the more pronounced the irregularity and prolongation of latency. The effect of GABA was especially marked in cases in which a sign of irregularity of latency was observed before the injection. None of these changes was noted with control injection of 0.9% saline solution (see Fig. 5).

The effects of GABOB was almost identical with that of observed with GABA, but there was some quantitative difference; while no change occurred in percentage frequency of CR after administration of GABA, a slight decrement of percentage frequency was noted 5 hours after or immediately after the injection of 25 to 50 mg./kg. GABOB without any behavioral changes (see Fig. 6). As an exception one of dogs did not show any decrement of percentage frequency by the injection of GABOB with a dose of 50 mg./kg. (see bottom diagram in Fig. 6).

The effect of GABOB upon discrimination was investigated. Following GABOB with a dose of 50 mg./kg. the positive CR in discrimination

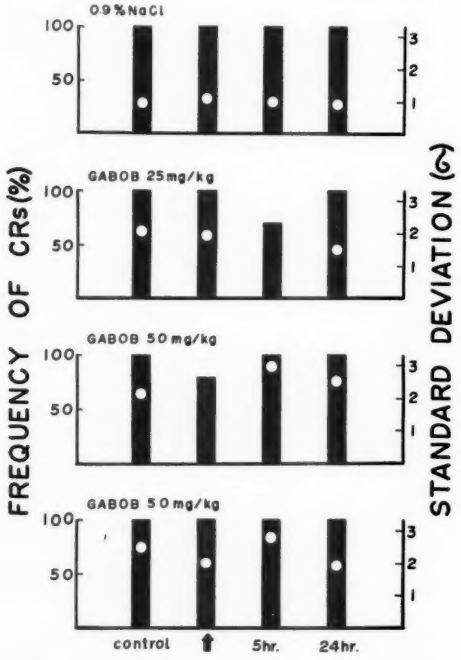

Fig. 6. Effects of intravenous administration of GABOB. Decrement of percentage frequency was observed. At bottom exception is shown in which no decrement of percentage frequency occurred following administration of GABOB.

was slightly inhibited immediately after the injection, did not occur after 24 hours and was found markedly inhibited even after 48 hours, although any other behavioral changes were not observed (see Fig. 7).

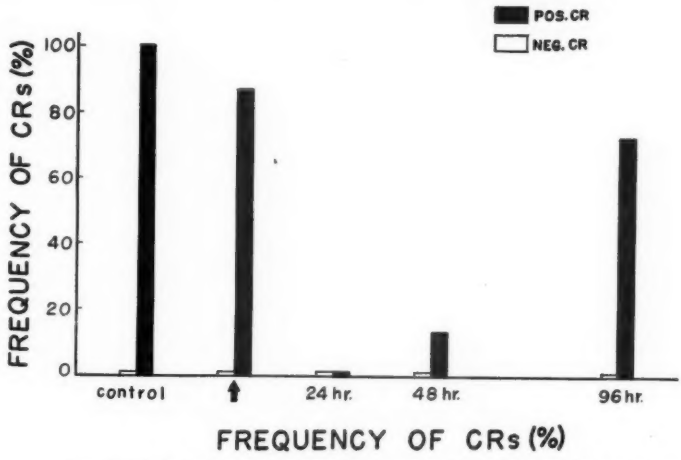

Fig. 7. Effect of intravenous administration of GABOB on discrimination. Positive CR was markedly inhibited (filled bar).

3. *Effects of GABA and GABOB administered intracisternally*

With intracisternal administration of GABA there were some differences from the effect of the intravenous injection. Following GABA in doses of 10 to 20 mg. some slight ataxia appeared, which recovered completely within about 20 min. With a dose of 10 mg. irregularity or prolongation of latencies could be observed immediately after the administration. With a dose of 20 mg. a slight decrement of percentage frequency and prolongation of latencies were observed. No changes were noted with control administration of 0.9% saline solution (see Fig. 8).

The effect of GABOB was also similar to that observed when GABA was applied intracisternally. After the administration the animals became depressive and was not so affectionate as before the administration. Some ataxia was also observed. The animals were apt to sink to the floor, although they showed interest in the environment. These behavioral changes were outstanding with a dose of 50 mg. Recovery from these abnormal behaviors was found to be complete within about 30 min. With a dose of 25 mg. a slight irregularity of latencies was noted without any decrement of percentage frequency. With a dose of 35 mg. a decrement of percentage frequency was observed immediately after the administration. With a dose of 50 mg. of GABOB no CR occurred immediately

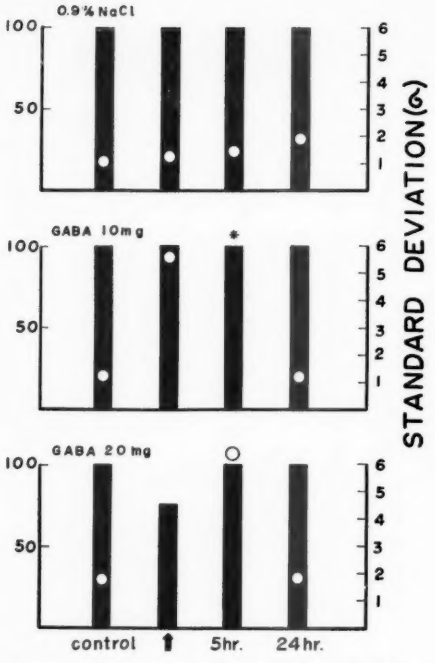

Fig. 8. Effects of intracisternal administration of GABA. *: not actually measured, but postulated.

after and 5 hours after the administration (see Fig. 9).

4. *Intracisternal application of GABOB in experimental neurosis*

One of the most interesting effects was that obtained by the intracisternal application of GABOB in experimental neurosis. Some animals showed chronic abnormal behavior, such as struggling or flinching, during the course of conditioning or after establishment of CR. The most prominent abnormal behavior was frequent tic-like flexions and sustained ones of the conditioned right hind leg without the CS. A slight autonomic discharge (vomitting, salivation and pupillary dilatation) was also observed. these abnormal behaviors were observed only in the experimental situation; the animals appeared normal outside the experimental room. Intracisternal administration of GABOB with a dose of 100 mg. ceased all such abnormal behaviors immediately after the administration.

Particularly the frequent spontaneous flexion of the right hind leg disappeared; the animals responded only to the CS and became relaxed and gentle. But on the following day the animals showed the same neurotic

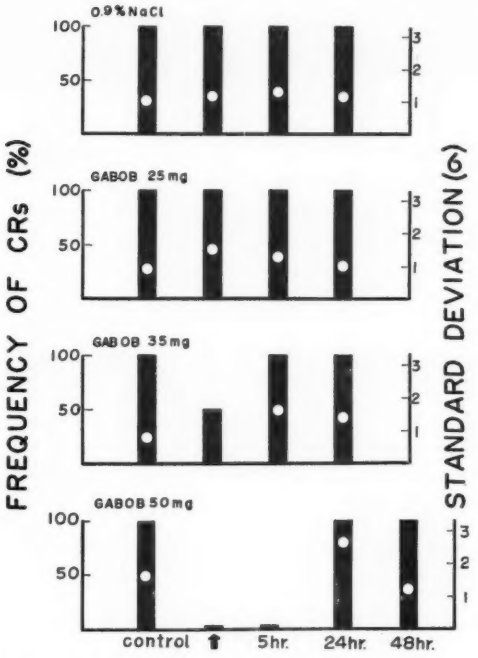

Fig. 9. Effects of intracisternal administration of GABOB.

signs as observed before the administration (see Fig. 10).

Discussion

As mentioned above, a prolongation of latency of CR observed during the course of acute extinction or discrimination may be considered as an expression of developing internal inhibition. As far as coditioned flexion responses in dogs, conditioned eyelid reactions in man, and conditioned knee jerks in man are concerned, the percentage frequency of response and amplitude has been said to have appreciably higher reliabilities than the latency,[4] but the present experiments have shown that the latency, especially its fluctuation, is a more sensitive index than the percentage frequency. It has been, however, reported by Shimizu[5] that in conditioning experiments on the rats the latency could remain unchanged in spite of a slight change of the central excitatory level, when the latter was extremely high.

From the results obtained by the administration of GABA or GABOB, it may be said that these substances contribute to the development of

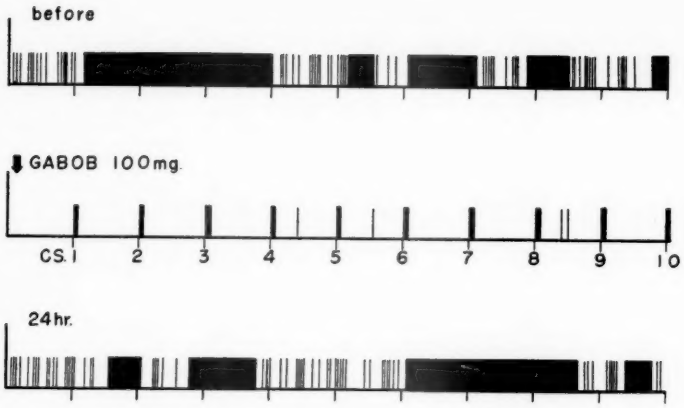

Fig. 10. Effect of intracisternal administration of GABOB on experimental neurosis. Vertical thin lines indicate spontaneous flexions of the right hind leg, and black rectangles sustained flexion. In middle diagram spontaneous flexions and those responding to CS are represented by thin lines and thick lines respectively.

Note disappearance or reduction of spontaneous flexions following GABOB administration.

internal inhibition. Between the inhibitory actions of GABA and GABOB no essential difference was noted. Judging from the results that the percentage frequency of CR decreased following both intravenous and intracisternal administrations of GABOB, but not following intravenous administration of GABA, the inhibitory action of GABOB might be slightly stronger than that of GABA. Hayashi[2] reported that the inhibitory action of GABOB was about 10 times as strong as that of GABA, judging from the critical concentration to stop a generalized seizure in dogs.

In the present experiments the effective intracisternal dose was about one tenth the effective intravenous dosis. In contrast to topical application of GABA, intravenous administration is said to produce neither central synaptic actions nor any detectable increase in brain concentration of the amino acid; in other words under ordinary circumstances GABA does not effectively penetrate the blood-brain barrier.[6] However, the effectiveness of intravenously administered GABA observed in the present experiment suggests that this agent penetrated the blood-brain barrier to some extent. After an intracarotid injection of GABA or GABOB Marrazzi[7] *et al.* and Hayashi[2] were able to observe the effects of these substances.

However, it is a question whether GABA or GABOB is a natural inhibitory factor in the brain, because the effective doses of these substances are too large in comparison with those of the known natural pro-

ducts like adrenaline or acetylcholine.

According to Pavlov, experimental neuroses develope only in animals of extreme types, either the extremely inhibitable or the extremely excitable. The animals observed in the present experiment appeared to belong to the latter type. It is of interest that the abnormal behavior observed in the experimental situation disappeared, though temporarily, following the intracisternal administration of GABOB.

Although there is no conclusive evidence to believe that GABA or GABOB is a natural inhibitory factor in the brain, it is of great interest that they are normal products of brain metabolism and show certain inhibitory effects upon the central mechanism of CR.

Summary

The effects of γ-aminobutyric acid (GABA) and γ-amino-β-hydroxy butyric acid (GABOB) on the avoidance conditioned reflexes (CR) in dogs were investigated. The results obtained as follows:

1. A prolongation of latency and decrement of percentage frequency of CR were observed during the course of acute extinction and discrimination.

2. Similar changes of latency and percentage frequency were observed following intravenous and intracisternal administration of GABA or GABOB. There was no marked difference between effective doses of GABA and GABOB.

3. The positive conditioned reflexes of discrimination were inhibited following the intravenous administration of GABOB.

4. The abnormal behavior observed in the experimental situation (experimental neurosis) disappeared, though temporarily, following the intracisternal administration of GABOB.

I wish to thank Prof. K. Motokawa for his invaluable discussion and suggestions throughout the course of the experiment and the preparation of the manuscript.

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Studies on Receptive Fields of Single Units with Colored Lights

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Introduction

The recording of spike discharge from a retinal unit or element, isolated by the microdissection or the microelectrode technique, has provided much information about not only the messages from the retina to the higher centers of vision, but also the neural mechanism within the retina itself.[1-8]

Hartline, who first described the receptive field of a single optic nerve fiber in the frog, showed that there were three main types of discharge, i.e. 'on', 'on-off' and 'off'.

In his experiment the discharge pattern was a fixed characteristic of each unit to the effect that it did not change essentially, whatever place within the receptive field of the unit might be illuminated.[1,2]

Later, Kuffler,[7] using the intact eye of the cat, showed that the discharge pattern of each single ganglion cell was variable depending upon whether the light illuminated the center or the periphery of the receptive field or both areas simultaneously.

He investigated the functional organization of the receptive field with a small spot of exploring white light and obtained some evidence which seemed to provide a neurophysiological basis of simultaneous brightness contrast. However, there seems to be no clue to the neurophysiological mechanism for color contrast.

This paper is concerned with the receptive field of the isolated single unit in the carp's retina and also the dependence of its response pattern upon the wave-lengths of the stimulating light.

Experimental

Method

The carp's inverted retina, mounted on a piece of black cloth soaked with saline, with the receptor side upwards, was exclusively used.

5 M NaCl-filled micropipettes, about 1 μ in tip diameter, were employed for recording, where a silver plate 1 $\times$1 cm. in size placed beneath the black cloth served as an indifferent electrode.

A recording electrode was inserted vertically in steps of 20 μ from the receptor side by the aid of micromanipulator. Since retinal ganglion cells are located in the innermost layer of the retina, attention was paid not to break the electrode tip by too deep insertion. The so-called ' S-potential ' (slow potential described by Svaetichin) was available for a measure of the depth of the electrode tip, because the structure responsible for the ' S-potential ' has been known from a number of histological controls to be situated in the layer closely distal to the ganglion cell layer.[9-12]

Thus, the recording microelectrode was advanced rather rapidly until it hit a ' S-potential ' and thereafter lowered so carefully that good isolation of a single unit might be possible. When the penetrating electrode could not seize any unit discharge as far as 100 μ after it encountered the ' S-potential ' ,it was withdrawn at once.

Usually unitary discharge of a ganglion cell was recorded in the depth of 200 μ measured with a microgauge attached to the micromanipulator.

Amplification of action potentials was performed with an A.C. amplifier with a cathode follower input stage. The time constant of the amplifier was first 0.1 sec., so that the ' S-potential ' could be recognized and then turned to 1.0 msec. for recording of the spike discharge.

The spikes usually obtained ranged from 100 μV to 250 μV in amplitude with a negative-positive configuration. Under favorable conditions they lasted for about 1 hour.

The general optical arrangement was the same as described in the previous paper.[13][14] An exploring light spot was 0.4 $\times$0.4 mm. in size and 6,250 lux in illuminance on the retina when no filter was placed in the light path. For monochromatic light stimulation fifteen interference filters were used, whose width of the transmission band was each less than 20 mμ. The energy of monochromatic lights was made equal by suitable neutral tint filters. Light stimulation was given every 5 sec., but recording was done at the later one of two successive exposures of the same monochromatic light to avoid any after-effect of the preceding light of different color.

Background illumination of suitable intensity was shone from another light source, when necessary.

All the experiments were carried out at room temperature of 15–17°C, unless otherwise stated.

Results

For convenience of description units which discharged impulses at ' on ', at ' off ' and both at ' on ' and ' off ' of a white test light centered on the tip of the recording electrode were designated as ' on '-center unit, an ' off '-center unit, and ' on-off '-center unit, respectively. Systematic studies were made on thirteen units which allowed sufficiently long-lasting explorations. Among these units eight were ' off '-center units, two ' on '-center units, and three ' on-off '-center units.

To begin with, records taken from one of the ' off '-center units are shown in Fig. 1. It is to be noted that the discharge pattern of this unit is

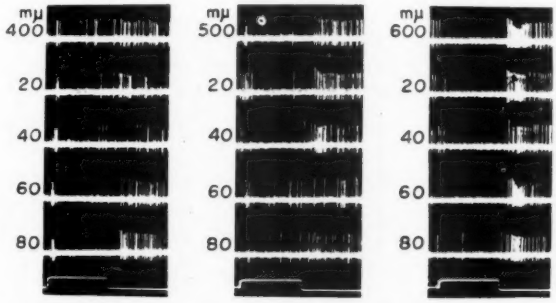

Fig. 1. Change of response type depending on wave-lengths. ' on ' type between 400 and 480 mμ, ' on-off ' between 500 and 580 mμ and ' off ' between 600 and 680 mμ. Time ; 0.1 sec.

variable depending on the wave-length of a stimulating light spot centered on the recording electrode. Within a range between 400 and 480 mμ this unit showed a burst of spikes at ' on ' which was followed by a silent period lasting throughout the duration of illumination and after the silent period normal spontaneous discharge returned without any sign of acceleration in the discharge rate. To the spectral lights of 500–580 mμ it responded both at onset and cessation of illumination with an acceleration of spike discharge. But the lights of wave-length longer than 600 mμ elicited only ' off ' responses without any discharge during illumination. Such relationship is shown diagrammatically in Fig. 2, in which the relative number of impulses (R.N.I.) is plotted as ordinates against wave-lengths as abscissae. The R.N.I. implied simply the number of impulses for 0.1 sec. from the onset of illumination for ' on ' responses, but for ' off ' responses it was represented by the number of impulses increased above the spontaneous rate ; it was so obtained that the average number of spontaneous impulses was subtracted from the number of impulses for 0.3 sec. after cessation of illumination. Black circles stand for the ' off ' responses and open circles

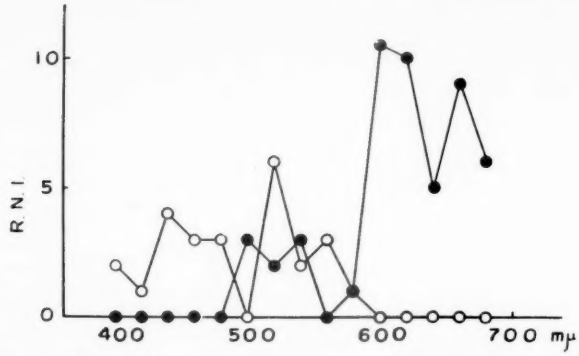

Fig. 2. Relative number of impulses (R.N.I.) of ' on ' response (open circles) and ' off ' response (solid circles) versus wave-lengths (reconstructed from Fig. 1).

for the ' on ' responses.

As can be seen in this figure, the type of discharge is ' on ' over the spectral range of 400–480 mμ, ' on-off ' between 500–580 mμ and ' off ' from 600 mμ on.

The question was raised as to whether such changes in discharge pattern were caused by changes in brightness or those in hue. So the effect of brightness was investigated with white light in the following experiment.

The effect of intensity

A single action potential of an ' off '-center unit was recorded on a fast sweep. It had a diphasic configuration of negative-positive sequence of 1 msec. in duration and 200 μV in amplitude.

According to Barlow's interpretation[15] such a diphasic potential originates in the soma of a ganglion cell or a very close portion of it. It is likely that a ganglion cell is situated at the center of its receptive field. From what has been said it may be assumed that the tip of the microelectrode was situated at the center of the receptive field in our experiments. In reality the response was found most active at the very tip of the microelectrode.

In a dark-adapted state, a small spot of the exploring white light of 0.4 ×0.4 mm. in size and 6,250 lux in illuminance was centered upon the site where the recording electrode was inserted. The intensity of this exploring light was reduced by means of neutral filters whose transmission factor was 0.7^n. The number of impulses discharged for 0.3 sec. after cessation of illumination was plotted as a function of the logarithm of intensity, and a S-shaped curve was obtained (Fig. 3). It is to be noted that the number of impulses decreases at very high intensities of illumina-

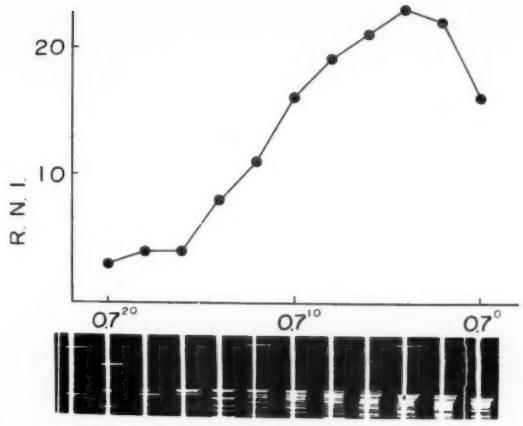

Fig. 3. Relation between relative number of impulses and log. intensity of white light with 6,250 lux as unity.

tion, and that the latency of the ' off ' discharge is obviously longer at such high intensities. Such an ' anomalous ' effect was observed by Kuffler,[7] too, who interpreted this effect in the light of receptive field organization.

It should be borne in mind that at least in this unit, changes in intensity or brightness caused no alteration in discharge pattern.

The receptive field of the unit

The receptive field of one and the same unit as used above was searched with a spot of white light 0.4 ×0.4 mm. in size which was displaced step by step along a straight line across the recording electrode. Fig. 4 shows the results obtained with a light spot of 6,230 lux in illuminance on the retina. When the tip of the electrode and its close neighborhood were illuminated, only an ' off ' type response was obtained. Illumination of a locus distant more than 1.3 mm. from the electrode produced an ' on-off ' type response. Under dark adaptation, with such a strong illumination the receptive field was so wide that no distinct boundary of it could be determined. It might be due to a considerable amount of scattered light.

When the intesity of the stimulating light was reduced to about 1/30 by a neutral filter, the receptive field of this unit was found to be as large as 3 mm. in radius. Thus the ' off ' center unit under consideration has showed an ' off ' center, an ' on ' surround and an ' on-off ' type of response in between, as Kuffler showed in the cat's retina[7] (Fig. 5).

Here it is to be noted that the receptive field of the isolated unit in the carp's retina is larger compared to those of frog's and cat's retinae which were estimated as large as 1 mm. in diameter.[2)6)7)8)]

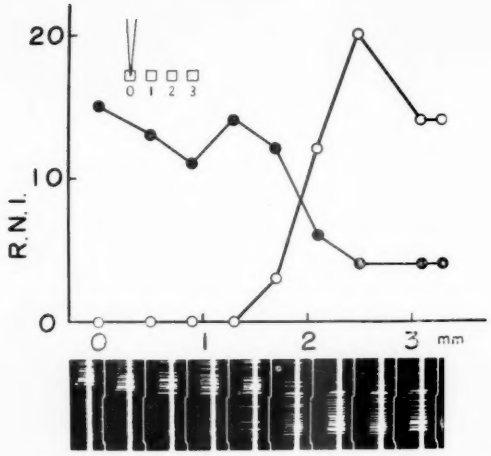

Fig. 4. Distributions of 'on' (open circles) and 'off' (solid ones) responses within receptive field searched with white light of 6,250 lux under dark adaptation. Positions of illumination relative to recording electrode are shown in inset.

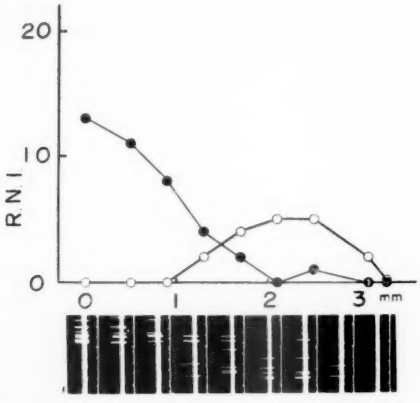

Fig. 5. As in Fig. 4, but with intensity reduced to 1/30.

Next, the receptive field of this unit was explored at various levels of background illumination with an intensity of the stimulating spot fixed at 6,250 lux. In Figs. 6 and 7 are shown the results which are almost similar to those obtained with the reduced intensity of the stimulating light.

Functional organization of the receptive field studied with colored lights

One and the same 'off' center unit was subjected to stimulation with

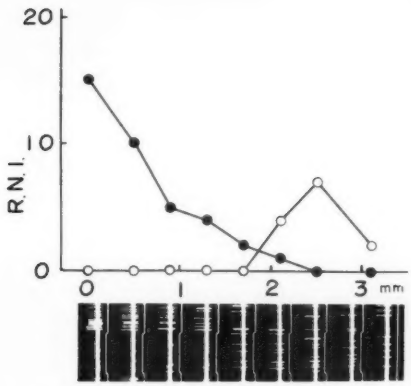

Fig. 6. As in Fig. 4, but under light adaption to 20 lux.

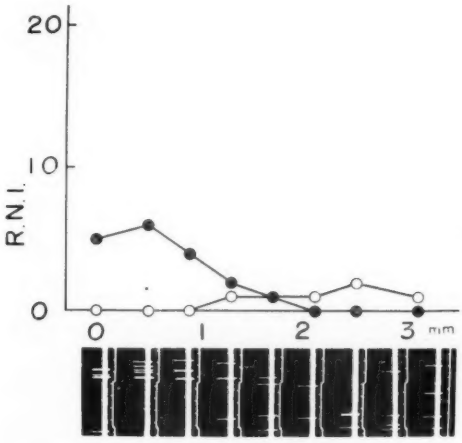

Fig. 7. As in Fig. 4, but under light adaptation to 300 lux.

a spot of colored light. Fig. 8 illustrates the findings obtained with the stimulating light centered on the microelectrode. The monochromatic lights of wave-lengths shorter than 540 mμ elicited ' on-off ' responses, while the other spectral lights evoked pure ' off ' responses. If the activity of the unit may be represented by the number of impulses during a given period, this unit is most active at the wave-length of 640 mμ with respect to the ' off ' response and at 480 mμ concerning the ' on ' response. These wave-lengths are nearly complementary to each other.

When the exploring colored light fell on the intermediary zone or

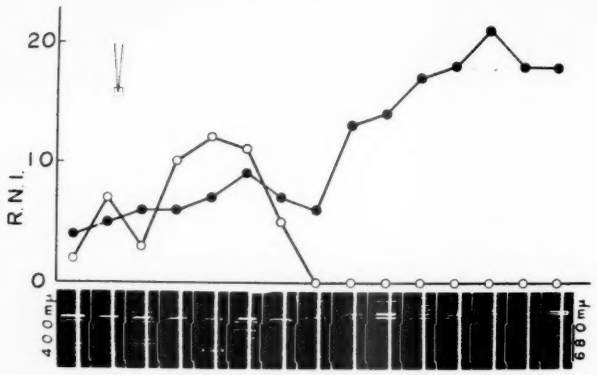

Fig. 8. Spectral response curves of 'on' (open circles) and 'off' (solid circles) responses at center of receptive field.

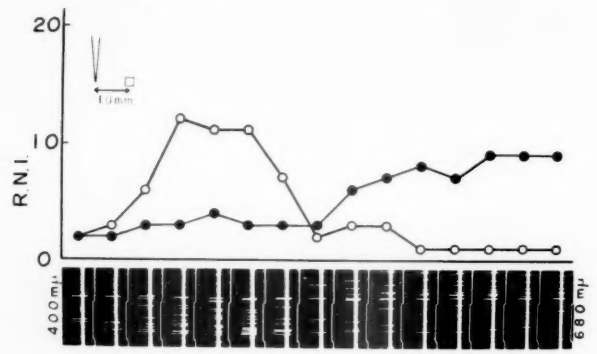

Fig. 9. As in Fig. 8, but at point 1 mm. away from center.

'on-off' area of this unit which was found with white light, the 'on' response appeared in the whole range of wave-lengths. Thus the response character of this unit showed the 'on-off' type (Figs. 9 and 10).

At the more peripheral area of the receptive field (2.0 mm. away from the recording electrode) any spectral light elicited only 'on' type response (Fig. 11). Throughout these experiments the wave-lengths which produced the most vigorous activity in this unit regarding 'on' and 'off' responses remained unchanged. Figs. 8, 9, 10 and 11 give wave-length dependence of responses at respective loci within the receptive field. From these figures the spatial distribution of response to each monochromatic light may be reconstructed. The results so obtained are shown in Fig. 12. The organization of the receptive field for the red light of 620 mμ is very similar to

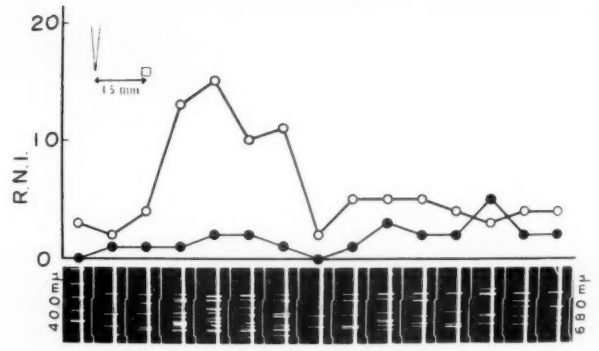

Fig. 10. As in Fig. 8, but at point 1.5 mm. away from center.

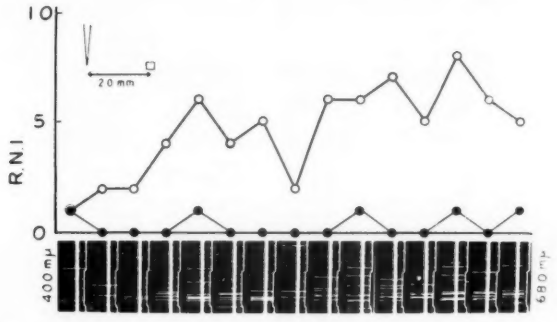

Fig. 11. As in Fig. 8, but at point 2.0 mm. away from center.

that obtained with white light (cf. Fig. 5), but very dissimilar to that for blue-green light of 480 mμ.

In some units the discharge pattern was fixed, depending on neither the wave-lengths nor the position of the stimulating light within the receptive field. An example is shown in Fig. 13. This unit was a pure 'off' unit. With diminishing intensities of monochromatic lights spectral range within which this unit could respond became narrower, until it was restricted to 600–640 mμ at the low level of 0.7^8 times the initial intensity. It seems that this unit is identical with Granit's red modulator.

Discussion

The present experiments have shown that the organization of the receptive field of a single ganglion cell in the carp's retina is very similar to that found in the cat's retina by Kuffler.[7] In addition, new and interest-

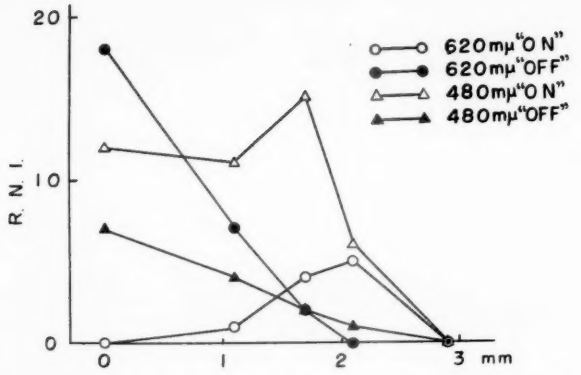

Fig. 12. Spatial distributions of 'on' and 'off' responses to red an blue-green lights within receptive field (reconstructed from Figs. 8–11).

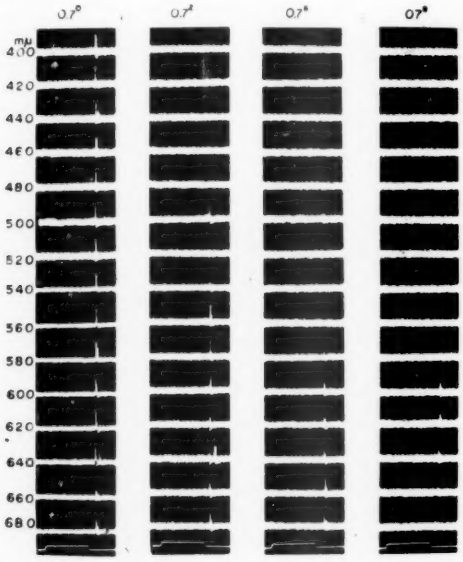

Fig. 13. Spectral response curve obtained from unit of fixed 'off' type. Ordinates; relative intensity. Abscissae; wave-length.

ing aspects have been disclosed that the type of discharge is dependent upon the wave-length of the light used for stimulation.

It has been known that a ganglion cell as a 'final common path' is subjected to complicated effects of facilitation and inhibition intermingled

by the retinal network intervening between the receptor and ganglion cell layers. Therefore, the type of response displayed by a retinal ganglion cell is nothing but a sum total of events occurring within the retina, so that the most dominant effect is a determining factor.

The fact that the organization of the receptive field searched with the red exploring light was very similar to that searched with the white light suggests the dominance of the preganglionic effect caused by the red component of the white light over those by the other components. This unit responded to the blue-green light of 480 mμ very actively, although the response type was quite different from that to the red light. In spite of such responsiveness to the blue-green light, the blue-green component of the white light must have been of little effect, because the discharge pattern elicited by the white light was very similar to that by the red light alone. It deserves attention that the effect of the white light could not be represented by the sum of the effects of all the spectral lights presented separately. From what has been observed above it may be supposed that the dominant effect inhibits the other effects. Although the neurophysiological mechanism of this inhibitory effect is not yet clear, it is certain that such a mechanism provides an explanation of some aspect of contrast.

Complementarity is the known characteristic of color contrast. The unit under consideration responded most actively to selected two portions of the spectrum, 480 mμ and 640 mμ (cf. Fig. 8) and these wave-lengths are nearly complementary, as stated above. The response type at the center of the receptive field of this unit was 'on' to the blue-green light, but 'off' to the red light, and thus reciprocal relation is obvious with respect to wave-lengths as well as response type. As has been shown by Kuffler[7] and confirmed by us, there is conspicuous reciprocity between the center and periphery of the receptive field. All these phenomena must be essential elements for the organization underlying contrast, successive or simultaneous.

No reciprocity was, however, found in the response of some elements whose response type was fixed. Such elements were described by Hartline in the frog, but not by Kuffler in the cat. It is likely that they contribute nothing to the phenomenon of contrast.

According to a personal communication from H. G. Wagner, E. F. MacNichol and M. L. Wolbarsht, they investigated the receptive fields of certain ganglion cells in the goldfish retina and obtained results very similar to ours. These fields were 'off' center, 'on-off' intermediate and 'on' periphery types. It is to be noted that the 'on' and 'off' processes could be light-adapted separately: Adaptation to a steady background of red light raised the threshold of the 'off' responses, but the threshold of the 'on' responses was slightly lowered. A blue adapting

light made the 'on' process less sensitive and the 'off' process more sensitive.

Summary

Receptive fields of single units in the carp's retina were explored with a small spot of white and monochromatic lights. Well-isolated single spike discharge 100–200 μV in amplitude was recorded with 5 M NaCl-filled micropipettes about 1 μ in tip diameter.

1. The dimension of the receptive field in the dark-adapted retina was as large as 3 mm. in radius.

2. When the response was 'off' at the center of the receptive field, it was 'on' at the periphery, and 'on-off' in the intermediary zone.

3. With white light three types of response pattern, 'on' center units, 'off' center units and 'on-off' center units were distinguished.

4. The type of response pattern was different according to the wave-length of the light used. For example, an 'off' center unit as determined with white light was also an 'off' center unit when searched with red light, but an 'on-off' center unit when explored with blue-green light.

5. In some elements the type of response was fixed throughout the receptive field and independent of the wave-lengths.

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Experimental Studies on the Central Hyperthermia

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Many cases of death have been experienced by excedingly high fever following excision of brain tumors or head injury. Such a central hyperthermia is one of the most fearful menaces to brain surgeons, and even in these days of all-round advancement in control measures during and after operations, still a number of cases of hyperthermia resisting all medical remedies are met with. Now, since Tscheschichin[1] reported on his study on the relation between surgical interference of the brain and the rise of temperature in animals, there have been many a work on the thermal or heat center, but there have been nearly no work on the study of the fundamental mechanism of central hyperthermia based on the results of observations of animals with experimentally induced central hyperthermia. So, the present author, as the first step of such an experimental study, made various attempts in inducing central hyperthermia by stimulating or injuring the generally accepted area of location of the heat center and introducing blood or foreign matter into the third ventricle, and succeeded in finding a method of immediate serviceability. In the following I will speak chiefly in this method, but by the way, I will make some comments on the localization of the thermal center on which opinions are still divergent, basing myself on the results obtained in the course of the experiments. Besides, the antipyretic effect of some autonomic blocking drugs[2] and adrenal cortex hormones[3][4] was also studied, using the animals with successfully induced hyperthermia.

Experimental

Material

In examining the change in body temperature under various surgical operations, rabbits showed fever by only a small-scale craniotomy or stimulation of any unspecified subcortical region, as much as the so-called heat center were stimulated, as shown in Fig. 1, so that they were in a very labile state against any surgical interference, in respect of their body tem-

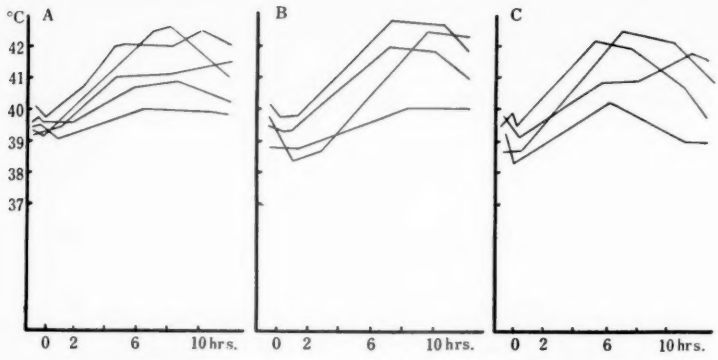

Fig. 1. Change of temperature of rabbits after various surgical interferences (measured after release from restraint). A: after puncture of the hypothalamus (the heat center?). B: after light puncture of the cerebrum. C: after small-scale craniotomy.

perature. Mongrel cats, when tied and fixed in extended position as the rabbits above, responded by rather lowered temperature to small-scale craniotomy, and the cat released from the fixed posture after the operation recovered its preoperative temperature in 4 hours. Thus, the cats showed a general stability against surgical attack, never showing any rise of temperature after the operation, as did the rabbits above (Fig. 2). Therefore, cats were used in my experiments throughout, as the best adapted for such a use.

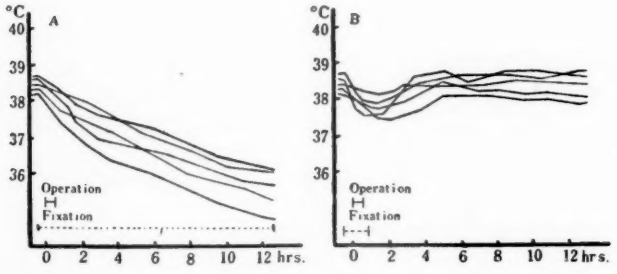

Fig. 2. Fluctuation of temperature of cats after small-scale craniotomy. A: as measured while keeping the cats tied after the operation. B: as measured after releasing them after operation.

Now, the normal rectal temperature of the cat has been reported variously as averaging 38°6′C (37° 5′ C～38°2′C) by Clark[5] as averaging-39°0′C (38°0′C～39°8′C) by Tanabe[6] and as ranging between 37°0′C and

39°5′C by Shirai.[7]

In my measurement, I used well domesticated cats and held them gently in arms, to prevent any effect of a forced position on their temperature, and obtained the values of 38°0′C～39°4′C or 38°7′C on the average, with the standard deviation of 0.35.

Since it is necessary for my experimental study to ascertain the upper limit of the daily fluctuation of the rectal temperature of the animals, a maximum thermometer was left inserted in their rectum for 24 hours while they were left untied, and the maximum temperature was found to stand at 39°1′C～39°3′C and in most of the cats remained below 39°2′C. Little seasonal fluctuation should be observed.

It is said that the rectal temperature is considerably affected by mere restraint and fixation, as well as by forced posture and surgical interf ference.[6][8][9] The following experiments were carried out to obtain informations on this point.

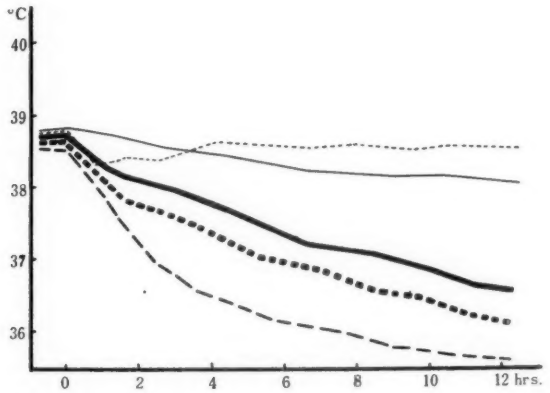

Fig. 3. Fluctuation of temperature of cat under restraint, fixation and surgical attack (mean values).
—— under fixation with the trunk and the limbs bent as far as possible (room temperature: 15°C–20°C).
—— under fixation with the trunk and the limbs extended fully (room temperature: 15°C–20°C).
– – ditto (room temperature: 8°C–10°C).
····· measured after release from fixation after small-scale craniotomy and exposure of bilateral carotid artery (room temperature: 15°C–20°C).
····· measured under fixation after the same operation (room temperature: ditto).

First, the 5 cats fixed in the prone position with their trunk and limbs fully extended at room temperature of 15°C～20°C showed a full of rectal

temperature by 0.5°C~1.0°C in 3 hours and by 1.5°C~2.5°C in 12 hours. When the atmospheric temperature was low, the drop was the more perceptible. But in the 5 cases fixed with their trunk and limbs bent as far as possible and the area of their skin directly exposed to the room air reduced to the minimum, the drop in the rectal temperature was very little at the room temperature of 15°C~20°C (Fig. 3).

In the 5 cats subjected to small-scale craniotomy or exposure of the carotid artery only, the drop in the rectal temperature was perceptibly larger than in the cases merely restrained and fixed, amounting to 1.0°C~1.5°C in 3 hour value and to 2°C~3°C in 12 hour value. The 5 cats released from fixation after small-scale craniotomy and light surgical operation recovered their preoperative temperature after a transient drop of 0.5°C~1.0°C 1 hour after the operation, and thenceforward showed a general stability in their temperature (Fig. 3).

Thus, the body temperature of cat being rather heavily subject to the effect of restraint and fixation, surgical intervention and the posture during the operation, we have to pay unfailing attention to this effect in experimental measurement of body temperature. In my experiments for determining the rise of temperature, such a drop will not cause any danger in overestimating the final results, but the opposite danger of a part of the rise being hidden under the drop is always present and care must be taken to minimise the effect as far as possible in preparing for the experiments.

Methods

Experiment I

Eighty-five normal cats without anaesthesia were fixed in the extended prone position with their head fixed with Horsley-Clarke's stereotaxic instrument.[10)-14)] Then, the anterior or posterior portion of their hypothalamus or the tuber cinereum was punctured with 22A-stainless steel[15)] needle of 0.5 mm diameter, or electrically stimulated or destroyed by electrocoagulation through a polyethylene-insulated 22A-stainless steel bipolar needle electrodes, or electric current was sent through the brain by application of a pair of silver electrodes of 1 cm. diameter on the skin of both the temples, and the change in their rectal temperature was measured at the room temperature of 15°C~20°C for 1 to 3 days, for comparative study. For electrical stimulation an AC of 50 c/s of 3 V was applied for 30 seconds—a current barely sufficient to cause convulsion in the animals. In some cases, a 50 c/s AC of 0.3 V was applied for 72 hours continuously. Of these 85 animals, 51 were left fixed after the operation and the other 34 were released from the fixation postoperatively, for subsequent observation (Table I). After the experiment, the punctured parts were electrically

coagulated in every case and excised for histological examination.

Experiment II

Ninety-six normal cats without anaesthesia were similarly fixed and small trepanation of only 2 mm. diameter was done at the median part of the parietal region ; a polyethylene tube of 1 mm. diameter with a side hole near its closed bottom was inserted through the cut hole gently into the third ventricle. After ascertaining the flow out and pulsation of the cerebrospinal fluid, 1.0 cc. each of one the following test materials were slowly introduced into the tube : 0.5% sodium citrate-added autogenous blood, autogenous whole blood, 50% suspension of autogenous blood cells in physiological saline, autogenous serum, autogenous blood denatured by boiling, 20% suspension of such denatured blood in physiological saline, 0.5% solution of sodium citrate in physiological saline, the pyrogen TTG (5 γ/cc. ; solution of a purified pyrogenic heteropolysaccharide isolated

TABLE I

Results of Stimulation and Destruction Experiments

Site affected	Test method	Cases kept fixed after operation		Cases set free	
		Total	Hyperthermic	Total	Hyperthermic
Hypothalamus, posterior portion	Bilateral puncture	5	0	5	0
Hypothalamus, anterior portion	Bilateral puncture	5	0	5	0
Hypothalamus, posterior portion	Unilateral electric stimulation 50 c/s, 3 V, 30 seconds	5	1	4	0
Hypothalamus, posterior portion	Bilateral electric stimulation 50 c/s, 3 V, 30 seconds	5	0	3	0
Hypothalamus, posterior portion	Bilateral electric stimulation 50 c/s, 0.3 V, 72 hours	6	0	3	0
Hypothalamus, anterior portion posterior portion	Bilateral electrocoagulation electric stimulation 3 V, 30 sec.	5	0	3	0
Hypothalamus, anterior portion posterior portion	Bilateral electrocoagulation electric stimulation 0.3 V, 72 hrs.	5	0	3	0
Tuber cinereum	Electric stimulation 50 c/s, 0.3 V, 72 hours	8	1	3	0
Tuber cinereum	Electrocoagulation	3	0	5	0
Total brain	Electric stimulation per cutem 50 c/s, 0.3 V, 72 hours	4	0	0	0

from a non-pathogenic bacterium Pseudomonas fluorescens in physiological saline), physiological saline and 5% and 20% glucose solution. After release from fixation the change in their rectal temperature was measured for 3 days.

TABLE II

Results of Infusion Tests

Method	Case number Total	Hyperthermic	Died
Infusion into the third ventricle			
Sodium citrate-added autogenous blood	21	18	3
Autogenous whole blood	4	4	0
Autogenous blood cells	5	5	0
Autogenous serum	4	4	0
Boiled autogenous blood	4	0	4
Ditto in 20% dilution in physiological saline	4	4	0
Sodium citrate	4	4	0
TTG	9	9	0
Physiological saline	4	0	0
5% glucose solution	4	0	0
20% ditto	4	0	0
Physiological saline infusion and pumping	3	2	0
5% glucose infusion and pumping	3	2	0
Autogenous blood into the substantia grisea of the 3rd ventricle,			
medial side	6	6	0
lateral side	6	0	0
Autogenous blood into the subcortical region of the parietal lobe	3	0	0
Ditto into the capsula interna	3	0	0
50°C physiological saline into the carotid artery	5	5	0

Besides, the results after 5 times of pumping following introduction of physiological saline and 5% glucose solution were also studied (Table II).

In the cases made feverish by introduction of autogenous blood, the introduction was repeated some times just after the pyretolysis or 72 hours after it and the rise and fall of their rectal temperature was followed up.

Experiment III

Twelve adult cats were similarly fixed, and 0.3 cc. each of autogenous blood was unilaterally introduced into the medial part (1.5 mm. lateral to the median line) or the lateral part (4 mm. lateral to the median line) of

the peripheral grey matter of the third ventricle or 1.5 cc. of autogenous blood was poured into the subcortical region of the parietal lobe or near the inner capsule, with the aid of the Horsley-Clarke's stereotaxic instrument.[10)–14)] After release from the restraint, the change in their rectal temperature was measured in comparison.

Experiment IV

Five adult cats without anaesthesia were fixed in the supine position, their common carotid arteries were bilaterally exposed, 10 cc. each of 50°C physiological saline was quickly injected per carotid and the wound was closed. Then the animals were set free and the change in their rectal temperature was measured for 5 days (Table II).

TABLE III

Efficacy of Autonomic Blocking Drugs and Hydrocortisone against Experimental Central Hyperthermia

Drug and method	Total	Case number Hyperthemic	Died
Cocktail M_1 injected intramuscularly (Chlorpromazine 1 mg/kg, Promethazine 1 mg/kg, Pethidine hydrochloride 2 mg/kg)	8	4	0
Ditto (double dosis)	8	0	1
Hydrocortisone injected intramuscularly	4	4	0

Experiment V

Twenty cats with central hyperthermia induced by the method described in Experiment II above were injected with Laborit's cocktail M_1[2)] (containing 1 mg/kg of chlorpromazine, 1 mg/kg of promethazine and 2 mg/kg of pethidine hydrochloride) intramuscularly in the femoral region every 6 hours, or the doses were doubled and besides 3 mg/kg of hydrocortisone was also similarly injected, and the change in their rectal temperature was followed up for 48 hours after the operation (Table III).

Results of Experiment I

Of the 10 cases punctured in the posterior portion of the hypothalamus bilaterally, 5 were kept fixed after the operation. In these cases the rectal temperature dropped by 1.5°C on the average in 3 hours. In the other 5 cases released from the fixation immediately after operation, the temperature fell by 0.5°C at once, but normalized in 4 hours. Not one of the 10 cases ever showed any rise of temperature throughout.

Of the 10 cases punctured in the anterior portion of the hypothalamus

bilaterally, (5 left fixed and 5 set free after the puncture), none ever showed feverishness and all the cases progressed similarly as the preceding group.

In the 9 cases with their posterior portion of the hypothalamus electrically stimulated with 50 c/s AC of 3 V for 30 seconds unilaterally, transient symptoms of mydriasis in the homolateral side, piloerection and convulsion were observable, but in the rectal temperature, of 5 cases kept fixed after the stimulation, 4 showed a drop of 2°C on the average in 6 hours, and fever was not registered (Fig. 4). In the other case, the temperature rose to 40°5′C in 24 hours (Fig. 5), and upon dissection, a hematoma of con-

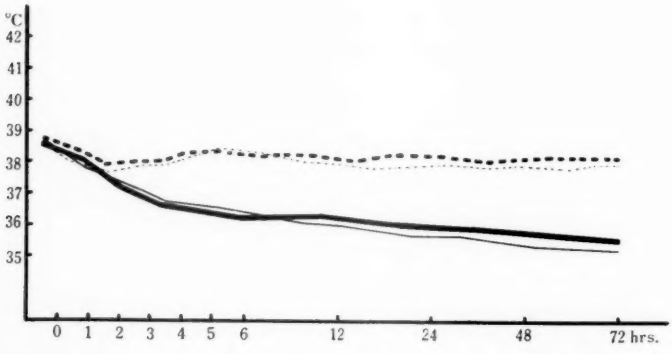

Fig. 4. Fluctuation of temperature after electric stimulation of the hypothalamus or the tuber cinereum (mean values).
—— under fixation after unilateral electric stimulation of the posterior portion of the hypothalamus (50 c/s AC of 3 V for 30 sec.), mean value of 4 cases free of hematoma in the stimulated site.
····· measured after release from the fixation after the same stimulation.
—— under fixation after electric stimulation of the tuber cinereum (50 c/s 0.3 V 72 hours).
····· measured after release from the fixation after the same stimulation.

siderable size was found in the peripheral part of the third ventricle (Fig. 6). In the other 4 cases released after the operation, the rectal temperature normalized in 4 hours, but none of them showed hyperthermia (Fig. 4).

When the same region was bilaterally stimulated, bilateral mydriasis, piloerection and convulsion appeared transiently, but in rectal temperature, the cases kept fixed after the stimulation showed a drop of 2°C on the average in 6 hours, while the cases released from the fixation immediately after the operation, the temperature fell slightly but stabilized at the preoperative temperature in 6 hours. In no cases rise of the temperature above the normal was registered.

The cases continuously given electrical stimulation by application

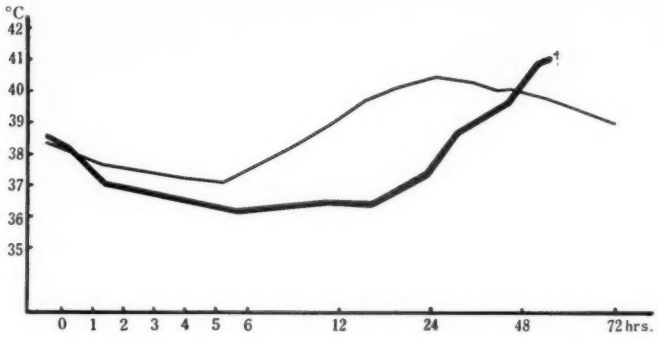

Fig. 5. Fever chart of two cases associated with hematoma in the stimulated site.
—— a case with the portion of the hypothalamus electrically stimulated with 50 c/s AC 3 V 30 sec. unilaterally, and kept fixed after the operation.
—— a case continuously given electrical stimulation by application of 50 c/s AC of 0.3 V for 72 hours and kept fixed.

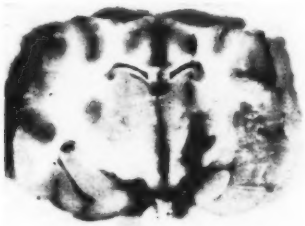

Fig. 6. Hematoma formed in the case with hyperthermia after stimulation of hypothalamus (frontal section of diencephalon).

of 50 c/s AC of 0.3 V for 72 hours bilaterally in the posterior portion of the hypothalamus showed no neurological symptom during the stimulation throughout. In the 6 cases kept fixed after the stimulation, the temperature fell by 2°C on the average in 6 hours, while in the 3 cases kept untied during the stimulation, the temperature dropped by 1°C on the average immediately after the operation but normalized in 6 hours. In no case, fever was registered.

Of the 8 cases with the anterior portion of the hypothalamus electrically coagulated and then its posterior portion stimulated with 50 c/s AC of 3 V for 30 seconds, the 5 cases kept fixed after the operation showed a drop of temperature of 2°C on the average in 6 hours, while in the 3 cases set free after the operation, the rectal temperature rose by 0.2°C on the average.

This, rise of temperature, however, is too small to be called fever.

When the stimulation was continued for 72 hours with the weak current of 0.3 V in the cases similarly treated, the temperature showed also a similar course of change, hyperthermia coming forth in no case.

In the 8 cases with the tuber cinereum stimulated from both sides with 50 c/s AC of 0.3 V for 72 hours in consecution and kept tied, no neurological symptom was observable during the stimulation throughout, the change in temperature was similar to that in the preceding cases, no pyrexia being observable, except in one case which died in 48 hours, showing the high fever of 41°0′C (Fig. 5). In this case, a rather wide-spread hematoma was found in the periphery of the third ventricle and in the base of the brain (Fig. 7). In the 3 cases similarly stimulated and set free, the rectal temperature dropped 0.5°C on the average immediately after the operation, then stabilized at the preoperative temperature in 6 hours and in no case fever was registered.

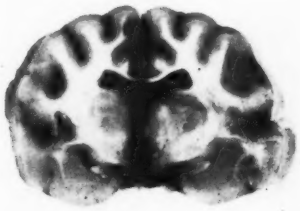

Fig. 7. Hematoma formed in the case with hyperthermia after stimulation of tuber cinereum (frontal section of diencephalon).

In the 8 cases with the tuber cinereum coagulated electrically, no neurological symptom was observed; in the cases kept fixed after the operation the temperature fell by 2°C on the average in 6 hours, in the cases left untied, the temperature slightly rose by 0.2°C on the average in 6 hours but soon stabilized thereafter at the preoperative temperature, high fever coming forth in no case.

In the 4 cases with the brain stimulated with 50 c/s AC of 0.3 V for 72 hours in continuance applied from outside of the skin and kept unreleased from the fixed posture after it, showed no neurological symptom and their temperature dropped by 0.2°C in 6 hours and kept falling off thereafter too.

All the above experimental animals were dissected after death to examine the injured sites, and it was found that in two cases alone that had fever of above 40°C, hematoma was found in the peripheral part of the third ventricle, as described above. These findings show that it is very

difficult to induce central hyperthermia by puncturing, destroying or otherwise stimulating only a definite point in the brain of cat, but perhaps such a fever may be induced by causing formation a bleeding focus of adequate size in the third ventricle or the base of the brain.

Results of Experiment II

In the cases with sodium citrate-added autogenous blood infused in their third ventricle, such neurological symptoms as mydriasis, erection of hairs and convulsion were observed transiently after the infusion, and in 18 of the 21 cases the temperature recovered from the drop occurring just after the operation in 3 hours and thereafter kept on rising above normal, reaching 39°8′C on the average in 6 hours and in 15 of them to 40°C～42°C in 24 hours (Fig. 8).

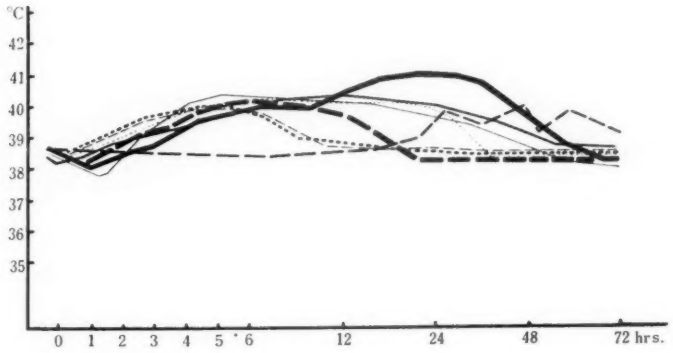

Fig. 8. Fever chart of experimental central hyperthermia by various method (mean values).
—— boiled and denatured blood in 20% dilution in physiological saline.
—— autogenous whole blood.
—— sodium citrate-added autogenous blood.
— — autogenous blood into the peripheral grey matter of the third ventricle, medial side.
— — 50°C physiological saline into the carotid artery.
"""""" pumping of the third ventricle.
— — sodium citrate.
····· TTG.

In the cases with autogenous whole blood infused in their third ventricle, pyrexia of 40°C on the average was observed in 12 hours in all the cases, though no symptom of excitation could be observed at the infusion (Fig. 9).

The cases infused with 50% suspension of autogenous blood cells in

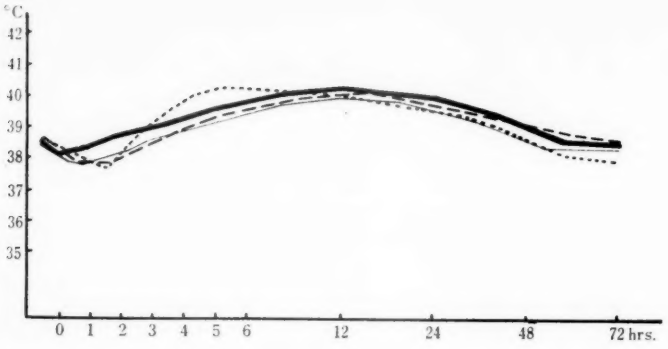

Fig. 9. Fluctuation of temperature of cats introduction of various components of autogenous blood into the third ventricle (mean values).
—— whole blood.
– – blood cells.
—— serum.
····· boiled and denatured blood in 20% dilution in physiological saline.

physiological saline showed no apparently anomalous symptom after the infusion and hyperthermia of 40°C on the average was observed in 12 hours in all the cases (Fig. 9).

In the cases with autogenous serum infused in their third ventricle, hyperthermia of 39°8′C on the average was observed in 12 hours in all the cases, though no excitation symptom appeared (Fig. 9).

The cases infused with autogenous blood denatured by boiling, showed no apparently anomalous symptom after the infusion, yet all died of excessive marasm but free of fever in 5 or 6 hours, but when the suspension was diluted with physiological saline down to 20%, the infused animals showed rise of temperature, up to 40°C in 6 hours (Fig. 9).

In the 4 cases infused with 0.5% solution of sodium citrate in physiological saline, such neurological symptoms as mydriasis, erection of hairs and convulsion were observed transiently after the infusion and hyperthermia of 40°C on the average was observed in 4 hours (Fig. 8).

When 5 γ of TTG was infused, no excitation symptom appeared at the introduction, but in 6 hours thereafter, all the cases had the fever of 40°C (Fig. 8).

In the cases when 10 γ/kg of the drug was applied by intravenous injection, the temperature never rose beyond 39°2′C, and when the dosis was raised to 50 γ/kg, only 2 of the 5 cases showed fever of 39°7′C 5 hours after the injection.

Neither excitation symptom nor hyperthermia could be observed

following introduction of physiological saline, 5% and 20% glucose solution, but when pumping was applied at the infusion, in all the cases transient mydriasis was manifest but no convulsion was observed ; 4 of 6 cases infused with one of the solutions showed considerable pyrexia, the temperature rising to 40°C on the average in 6 hours (Fig. 8).

When the cats that showed fever upon infusion of autogenous blood, the infusion was repeated immediately or 3 days after the defervescence, a fever taking a course similar to that after the first infusion occurred again and thrice upon the first and the second reinfusion.

Results of Experiment III

Hyperthermia was induced in all the cases with the medial part of the grey substance in the periphery of the third ventricle unilaterally with autogenous blood without exception, the temperature rising to 40°C on the average (Fig. 8), but fever was never induced by infusion of the same into the lateral part of the grey substance of the third ventircle or into the subcortical region of the parietal lobe or near the internal capsule.

Results of Experiment IV

All the cases injected with 50°C physiological saline in the carotid artery had fever rising to 39°8′C in 24 hours and remittent fever of 39°5′C to 40°3′C followed for 4 days thereafter. No anomalous symptom could be seen directly after the infusion, but with the onset of fever about 24 hours afterwards, the animals showed slackened activity and response, but the fever soon began to abate gradually and none of the animals succumbed to the hyperthermia (Fig. 8).

Results of Experiment V

When the cats with central hyperthermia induced by introduction of autogenous blood into their third ventricle, as described in Experiment II above, were intramuscularly injected with Laborit's cocktail M_1 for obtaining informations on its efficacy in inhibiting hyperthermia, 4 of 8 cases tested showed no fever higher than 39°2′C, but in the other 4 cases hyperthermia of above 40°C was observed. When the dosis of cocktail was doubled, none of the 8 cases thus tested showed fever above 39°1′C, but 6 of them died within 12 hours (Table III).

When such cats with central hyperthermia were given hydrocortisone, all the 4 cases thus tested suffered from hyperthermia over 40°2′C (Table III).

Comment and Summary

The fast advance of modern brain surgery since the beginning of the

current century has brought solution to many a difficult problem, and yet all the efforts of many workers in this field have failed to arrive at definitive answer on many points. One of these unsolved questions is the question of hyperthermia often experienced after brain surgery. In reviewing the past workers on this question, we find there are not a few reports on experimental hyperthermia[6)16)-41)] induced by puncture or injury, in various parts of the brain since the days of Ott[16)] and Aronsohn & Sachs,[17)-19)] but in most of these studies, rabbits were used as the experimental animals, but rabbits are so labile in their body temperature and the experiments were carried out with so little attention to the change in temperatures, (which by the way amounted only a rise of 0.2°C~1.0°C) induced merely by the restraint and the surgical interference on the animals, we feel justified in discounting the value of such works. Under such a circumstance, it is natural that there has been no generally acceptable solution on the problem of the exact localization of thermal center.

Since many cases have been reported in which no hyperthermia was induced following unerring introduction of the puncture needle into sites described as the location of the thermal center,[46)] there have a considerable number of authors who have expressed doubt on the presence of a " thermal center " at all.[42)-49)]

Now, for the study of a method for treating postoperative hyperthermia, it is indispensable first of all to succeed in inducing central hyperthermia in experimental animals. It is, however, a very difficult proposition to produce such animals with central hyperthermia, seeing that the problem where the pyrogenetic center is exactly located remains yet unsolved, and in the copious literature on the subject, I could not find a single report of 100% success in producing such animals, most of the authors merely confessing the difficulty of the experimental production.[6)49)] So, the present author was induced to make the attempt of producing central-hyperthermal cats by application of a variety of surgical methods.

As the sites pointed to as the possible pyrogenetic center, we may enumerate the pons,[17)] the upper part of the corpora quadrigemina,[20)] the thalamus,[21)-24)] the corpus striatum,[19)21)23)-31)] the periphery of the third ventricle etc.,[32)-35)] but more recently, the hypothalamus[5)6)32)36)-39)] and the tuber cinereum[16)40)41)] have come more into attention in this connection, the former being said to contain the heat-radiation center in its aterior portion and the pyrogenetic center in its posterior portion.[38)] Therefore, I first tried puncture, electrical stimulation and electrocoagulation in these parts, but hyperthermia over 40°C followed in only 2 of the total 85 cases, and I was convinced that mere simple stimulation or destruction of these parts cannot induce hyperthermia. During this series of experiments, however, I found hyperthermia induced in 2 cases, in either of which a

rather large hematoma was found spreading from the third ventricle into its surrounding tissue, in contradistinction to all the other non-hyperthermic cases.

Accordingly, I concluded that a wide-scoped stimulation of the periphery of the third ventricle is effective in causing hyperthermia and proceeded to the experiment of introducing sodium citrate-added blood directly into the third ventricle and found hyperthermia induced in nearly all the cases. For ascertaining what substances are effective in causing fever, sodium citrate, whole blood without sodium citrate, blood cells, serum, boiled autogenous blood and some other stimulant foreign matters were similarly infused and hyperthermia was successfully induced in almost cases to the level of 39°8′C～42°0′C (a rise of temperature by 1°C～3°C). When physiological saline or 5% glucose solution was infused, no hyperthermia was caused, but when pumping out of the infused matter immediately followed the infusion, considerable pyrexia occurred in some cases, and it was proved that mechanical stimulation applied to the periphery of the third ventricle was also effective in causing hyperthermia.[50),51)]

The above findings suggest that the hyperthermia is not induced by tampering with any one specific point in the brain but by stimulation of a wide-spread area in the grey matter in the periphery of the third ventricle. For nearer information on what portion in the grey matter around the third venticle is most sensitive in this respect, autogenous blood was introduced into one side of the medial part of the grey matter and fever over 39°8′C followed upon blood infusion into the anterior, the middle and the posterior portions of the periventricular grey matter, but no pyrexia was observed following infusion into the lateral portion. Thus, it was indicated that all the medial side of the grey matter around the third ventricle induces hyperthermia under stimulation. In this way, I succeeded in producing experimental hyperthermia with passable certitude, but with the aim of ascertaining how much cerebral edema had to do with hyperthermia, I made the further experiment of injecting 20 cc. of physiological saline warmed to 50°C through the carotid artery and found hyperthermia induced in all the cases. This is perhaps due to the edema spreading to the medial side of the grey matter around the third ventricle.

In the above experiments, pyrexia came forth the soonest in the cases of pumping following infusion of the third ventricle, followed by the infusion of sodium citrate, TTG, boiled autogenous blood, whole blood and blood components in the order named, pyrexia being most retarded following injection of 50°C physiological saline into the carotid. The induced hyperthermia lasted for the shortest following pumping and infusion of sodium citrate applied to the third ventricle, abating in about 6 hours, while following infusion of blood components, boiled autogenous blood and

pyrogenetic substances, the fever lasted for about 40 hours; upon intracarotid injection of physiological saline the hyperthermia continued intermittently for 4 days. The level of fever was the highest after infusion of sodium citrate-added autogenous blood, next after pyrogenetic preparations, boiled autogenous blood, whole blood, blood cells and serum in the order named, but the difference was always slight, the rectal temperature never coming off the 40°C mark (Fig. 8 and 9).

Ninomiya[52] of this clinic recently reported on his clinical observations on brain tumor cases and emphasises the finding that postoperative hyperthermia occurred in high grade and frequency in the cases with the tumor in the periphery of the third ventricle, when a large quantity of blood had run into the ventricle at the operation, when brain edema was severe and especially when the herniation of brain was present or the intracranial pressure was very high. These clinical experiences may be cited as valuable grounds endorsing my experimental findings, as well as giving some hints for studying measures for preventing and treating postoperative hyperthermia.

Autonomic blocking drugs and hydrocortisone were administered to the central-hyperthermic animals thus produced for studying their therapeutic efficacy. It was found that not very much could be expected from mere application of such drugs alone, but if adequate attention is paid to adjust the dosis and the method of dosage, these may be effective in defending the vital organism from excessive stress and offering the opportunity of its recovery. It is my ambition to search for such methods of treating hyperthermia., using my experimental hyperthermic animals.

Conclusion

1. Generally speaking, mammals show a tendency to gradual fall of their body temperature when restrained and fixed, especially markedly when they are fixed with their trunk and limbs fully extended, the environmental temperature is low or surgical interference is applied in the fixed position. Therefore, experiments concerned with temperature should be carried out under unconstrained position as far as possible.

2. The fluctuation of body temperature is widely different by species of animals. For example, rabbits are often subject to high fever following very sliglt stimulation. Such temperature-labile animals are not to be used in experiments in which the body temperature is an important item. Cats, however, were found fairly stable in their temperature, and the author selected 269 mongrel cats in total for experimental purpose.

3. The different parts of the hypothalamus and the tuber cinereum lately in the focus of attention as the thermal center were stimulated or damaged by a variety of methods, but hyperthermia of above 40°C could

not be induced except in two of the 85 cases tested. In these 2 cases, a rather large hematoma was found in formation in and around the third ventricle. It was inferred that a surgical stress to a small area in this part is not sufficient to produce hyperthermia.

4. When autogenous blood or stimulative substances were directly introduced into the third ventricle or mechanical stimulation was applied to the wall of the third ventricle, hyperthermia was successfully induced in 52 of the 61 cases (86%), 7 of them died (11%) and 2 only survived free of hyperthermia (3%). When it was the medial side of the grey matter around the ventricle that was subjected to the infusion, hyperthermia was induced without exception, but then it was its lateral side, no hyperthermic case appeared subsequently. The above findings seem to show that the nuclei around the third ventricle, in particular, those on the medial side of the ventricle, cause hyperthermia under stress by joint action.

5. Hyperthermia followed also injection of 50°C physiological saline through the carotid artery. This is perhaps due to the wide-scale brain edema induced thereby affecting the nuclei around the third ventricle and causing fever.

6. The efficacy of autonomic blocking media and hydrocortisone against experimental central hyperthermia was also studied.

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Cardiac Response to Local Cooling in Man

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Introduction

It has been well known that the heart changes its rhythm responding to sensory stimuli. In the authors' laboratory Kato[1] investigated in rabbits and dogs cardiac accelerations caused by acoustic stimuli, and Hoshi *et al.*[1] studied in rabbits the bradycardia which was brought about by mechanical and chemical stimulation of nasal mucosa.

Among various modalities of cutaneous sensations, the sensation of pain is such that most frequently provokes emotional and adaptive-protective reactions involving somatic as well as autonomic nervous system and its end-organs. Consequently pain must exert strong influence also upon the cardiac activity.

Present study aims to investigate the cardiac response to cutaneous stimulation in man and to clarify some of the underlying physiological mechanisms concerned. Most of the direct stimuli utilized for eliciting the sensation of pain in the skin are really harmful and can not be applied continuously to the subject for more than a few minutes. The change of cardiac rate, on the other hand, sets in rather slowly; consequently, to observe the whole course of cardiac response to pain it is needed an adequate method of arousing and sustaining the sensation of pain without injuring the subject. As the local cooling of the skin causes pain when it exceeds a certain limit, it seems to be most suitable for the above purpose, and was used throughout the present investigation.

Hines & Brown's "Cold pressor test"[3] has been generally known as a measure of the reactivity of blood vessels, particularly to detect a disposition to the essential hypertension. According to some authors[4-5], however, the phenomenon is nothing more than an expression of individual sensitivity to *pain* elicited by intense cold. Taking such an idea into consideration the blood pressure was also measured in the experiments along with the recording of the cardiac rate.

Experimental

Methods

The subjects studied were 8 healthy persons, 7 males and 1 female, their age ranging from 23 to 36 years.

The experiments were performed from April 1956 to March 1957. The room temperature recorded was from 8 to 25°C.

The subject was kept in complete rest and ease, and in recumbent posture except when the test stimulus was applied to the foot. In such a case the subject was placed in a reclining position. In winter the body was kept in adequate warmth being wrapped with blankets, hot water bottles and other conventional heating devices if necessary.

Local application of cold to the skin was performed as follows: a hand or a foot of the subject was dipped into the cold water in a crock placed close to the bed. The quantity of the water was about 5 liters, and the temperature was adjusted for each experiment. During immersion of the hand or foot the water was continuously stirred with a glas rod to insure the constant and uniform cooling of the extremity.

Changes in cardiac rate was recorded photographically to all its instantaneous variations by means of Matsuda's "Cardiotachograph"[6-7]. Its new type enables to record the instantaneous changes in cardiac rate exactly as a succession of dots or short horizontal bars. The blood pressure was taken in the arm of the opposite side every 30 seconds by the use of a conventional mercury sphygmomanometer. The skin temperature of the immersed hand or foot was recorded with a copper-constantan thermocouple which was fixed on the pad of the distal phalanx of the thumb. In addition, respiratory movement was also recorded by means of a pneumograph on the same photographic paper.

Before each experiment of immersion, the hand was dipped into the water of 37°C for 3 minutes or more as a control. Though 37°C is definitely above the "normal" skin temperature, it is well within the indifferent range of temperature as will be shown later.

Results

I. Responses to simple cold stimulus

When the subject's hand was immersed in cold water at various temperatures for 2 to 10 minutes the changes in cardiac rate and other manifestations were as follows:

(i) *Cardiac rate:* By preliminary experiments it was ascertained that no change in cardiac rate occurred on immersing the hand in water of between 37° and 12°C, but at temperatures 10°C or below a certain characteristic change of heart rate was observed. The lower the tempera-

ture, the greater was the change. Forty-one tests were carried out on 6 subjects, having their hands immersed in water at 5°C, for 10 minutes. An example of such experiments is shown with the actual records in Fig. 1 and in diagram in Fig. 2.

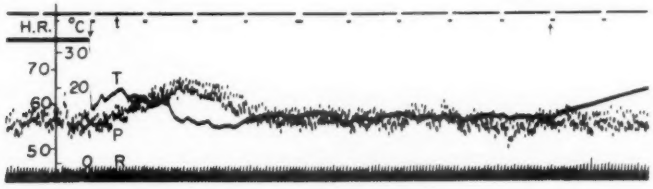

Fig. 1. Change in cardiac rate on immersing the hand in cold water at 5°C for 9 minutes.

↓↑: Signals of immersing and removing the hand.

t : Time signal, every minute.

T: Skin temperature of the immersed hand at the tip of its thumb. Calibration in °C on the left scale.

P: Cardiotachogram, (instantaneous cardiac rate), its calibration scale on the left in heart rate per minute (H.R.).

R: Respiratory movement, inspiration upward.

The average course of response was as follows: the cardiac rate started to increase almost immediately or within 25 seconds after the dipping, and reached its peak value from 60 to 100 seconds after the immersion. Following the peak, the cardiac rate, in spite of the continuation of immersion, began to decrease and gradually returned to the control value at about 4 to 5 minutes after the immersion. (In instances where the water temperature was extremely low such as nearly 0°C, cardiac rate failed to return sufficiently close to the initial level within the time interval as above stated, i.e., it remained fairly long at the accelerated level).

The increase of cardiac rate at its peak of change was maximally 29 per minute, on the average 14 per minute.

In about one third of the cases a bradycardia was observed following the initial tachycardia during the immersion. When the immersion was discontinued in 2 to 3 minutes the cardiac rate returned to control level sooner than when the hand had been dipped longer.

The quantitative relationship of increase of cardiac rate versus water temperature was investigated. Figs. 3 & 4 illustrate the results of such experiments. From Fig. 4 it can be seen that the heart rate increase was a negative linear function of the logarithum of the water temperature.

(ii) *Blood pressure:* Immersing the hand in cold water below 12°C caused an elevation of blood pressure, systolic as well as diastolic. As indicated in Fig. 2, the change of systolic and diastolic pressures paralleled

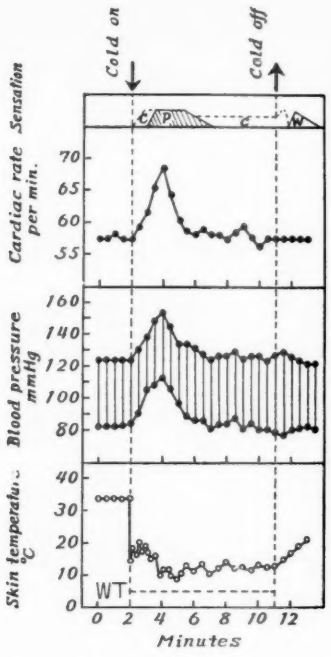

Fig. 2. Changes of cardiac rate, blood pressure and skin temperature on immersing the hand in water at 5°C for 9 min. Same case as of Fig. 1.
WT: Water temperature. Upper: subjective experience of sensation, diagramatically. C: Cold sensation. P: Pain sensation. W: Warm sensation.

closely the change of cardiac rate.

The average rise of blood pressure when the water temperature was 5°C was systolic 25 mm., and diastolic 26 mm Hg. There was a statistically significant correlation between the increase of cardiac rate and the riase of blood pressure.

(iii) *Respiratory rate:* Immersing the hand in cold water at 5°C induced in about half of the cases an acceleration of respiratory rate by 2 to 5 per minute within 1 to 2 minutes of immersion.

Though the cardiac acceleration due to local cooling was more remarkable in cases with respiratory acceleration, the change of the cardiac rate was not parallel with that of the respiratory rate. There was observed no significant correlation between the original respiratory rate and the increase of cardiac rate.

(iv) *Skin temperature:* As shown in Figs. 1 and 2, the skin tempera-

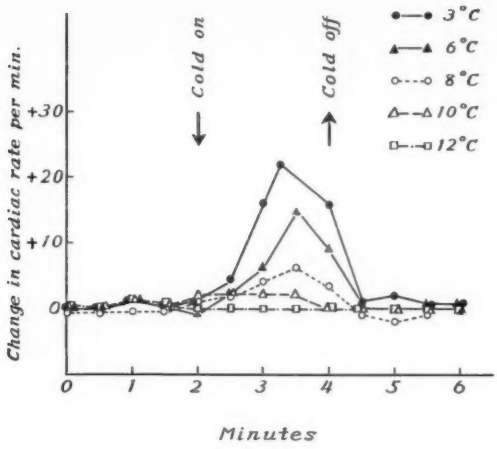

Fig. 3. Changes in cardiac rate produced by immersing the hand in water at various temperatures for 2 minutes.

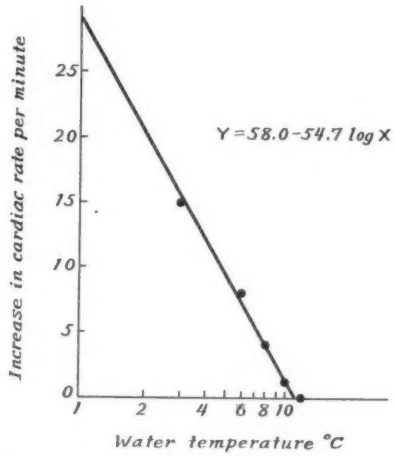

Fig. 4. Relation between water temperature and change in cardiac rate. The data in Fig. 3 are plotted. Abscissa: Water temperature in logarithmic scale. Ordinate: Change in cardiac rate per minute.

ture dropped at first fairly suddenly to about 10°C upon immersing the hand in cold water at 5°C, then fell down rather slowly, with more or less irregular fluctuations, to reach its minimum value approximating the water temperature or a few degrees above it. This was about 4 minutes

after the immersion. However, a few more minutes later the skin temperature rose a little (by 1 to 3°C) and remained at about the same average value as long as the cooling was maintained. On withdrawal of the hand from the cold water the skin temperature returned to the original level very gradually. Or in some cases it did not return completely for a while.

(v) *Cutaneous sensation and pain:* The cold sensation experienced, of course, from the beginning spread itself soon from the hand toward the upper arm and shoulder. About 40 seconds after the immersion an aching appeared in the fingers, which became rapidly aggravated in intensity and extended to the trunk, at times with an intensity hardly bearable. And the subject complained frequently of generalized tension, precordial aching even to agony, pallor of face, cold sweating, nausea and uneasiness in abdomen with augmented peristalsis.

All of these symptoms and signs, however, tended to subside spontaneously in spite of the persistence of the cold stimulus, and the aching which had spread to the shoulder and chest became localized again to the fingers, then distinctly milder leaving only dull sensation of cold in the immersed hand.

On withdrawal of the hand the cold sensation got temporarily intensified, then it was replaced by a sensation of warmth ascending from the fingers. At the same time there was often experienced an pulsating pain for a few minutes. Following subsidence of these sensations normal state of sensory indifference was resumed.

The above finding clearly indicates the existence of " adaptation " in the cardiovascular as well as sensory effects of cold stimulus applied locally to the skin.

In view of the apparent correlation between the pattern of the subject's sense of cold, warmth or pain and that of the skin temperature which is considered to reflect the sate of local circulation, the influence of local obstruction of circulation upon the effects of cold was further studied.

II. Local cooling combined with obstruction of blood flow

In the first place it was experimentally ascertained that, when the blood supply to an arm was interrupted for 10 minutes by inflating a sphygmomanometer cuff around the upper arm with a pressure about 20 mmHg. higher than the systolic pressure, no change was produced in cardiac rate as well as in blood pressure though the skin temperature of the hand decreased by 3 to 5°C and the subject experienced mild sensation of cold. No pain was caused at all.

Then the experiment was conducted as follows: Following the occulusion of circulation to the hand as described above for 3 minutes the hand was immersed in cold water, and the changes in cardiac rate and other manifestations were studied. One of the results is illustrated diagramatic-

ally in Fig. 5. The changes in cardiac rate, blood pressure and skin temperature taking place on immersing the hand persisted as long as the local circulation was occluded, i.e., they did not undergo " adaptation " as it did otherwise. On releasing the pressure in the cuff the cardiac rate and blood pressure turned to decrease keeping pace with the slight but distinct rising of skin temperature notwithstanding the continuous exposure to cold of the hand.

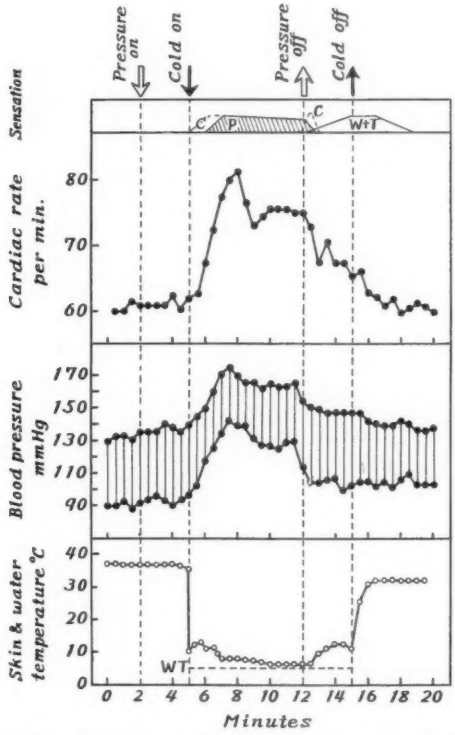

Fig. 5. Effect of circulatory obstruction and cold stimulation on cardiac rate, blood pressure, skin temperature and sensations of the subject. After the circulation to the hand had been stopped by means of a pneumatic armlet, the hand was immersed in water at 5°C. C: Cold sensation. P: Pain sensation. W: Warm sensation. T: Pulsatory pain. WT: Water temperature.

Beside these tests, in some cases the blood supply to the hand was interrupted after the hand had been cooled in the water for 3 minutes (Fig. 6). In such cases also, the skin temperature remained steady at its lowest value and the changes in cardiac rate and other manifestations

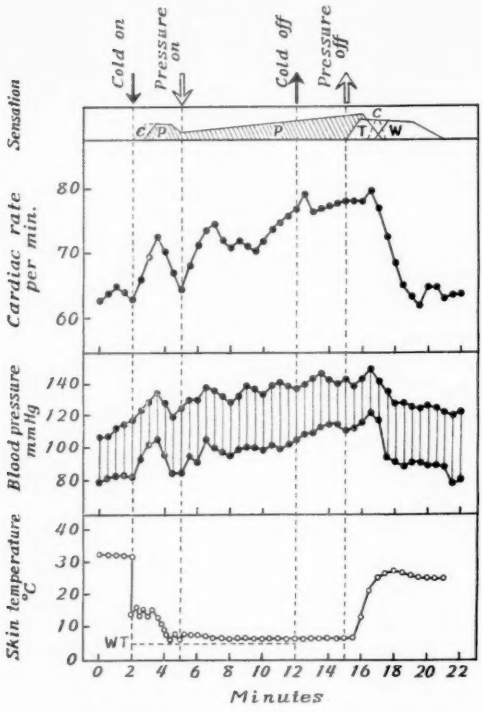

Fig. 6. Effect of stimulation and circulatory obstruction. After the hand had been immersed in cold water (5°C), the circulation to the hand was stopped. See explanations to Fig. 5.

persisted without any sign of adaptation throughout the persistence of circulatory obstruction and cooling. Noteworthy is the fact that even after withdrawal of the hand out of the cold water the increased cardiac rate and blood pressure value as well as the lowered skin temperature did not tend to return as quickly as in the case without circulatory stasis if the pressure-cuff was not released. In short, no adaptation in the response to cold stimulus was observed so long as the blood supply to the cooled area was stopped.

III. Spatial summation

The effect of the size of the immersed areas of the skin upon the cardiac response was investigated. On the same day and in the same subject a series of experiments was performed in which the several parts of hands or feet were exposed to cold water in combination, simultaneously or successively, e.g., one to several fingers of a hand, right and left hands, or left

hand and right foot, and so on. The results obtained are summarized in Tab. 1. Figure 7 represents the actual records of " cardiotachogram " in one of such experiments.

TABLE I

Spatial Summation in Cardiac Reaction to Cold Applied to Several Areas of the Limbs

a) When several areas were stimulated stimultaneously.

(Subject I. M.)

Cooled part	1 finger	3 fingers	4 fingers	hand
Increase in cardiac rate for 3 min.	6.7	21.5	27.8	45.0
	(1.0)	(3.2)	(4.1)	(6.7)
Area	(1.0)	(3.0)	(4.0)	(7.0)

(Subject K. T.)

Cooled part	left hand	right hand	both hands
Increase in cardiac rate for 3 min.	3.9	5.8	11.5
	average 4.8		
	(1.0)		(2.4)
Area	(1.0)	(1.0)	(2.0)

(Subject M. N.)

Cooled part	right foot	left hand	right foot & left hand
Increase in cardiac rate for 3 min.	9.6	9.8	18.4
	(1.0)	(1.0)	(1.9)
Area	(1.0)	(0.6)	(1.6)

b) When several areas were stimulated succesively in the order indicated.

(Subject M. N.)

2 fingers →	5 fingers →	hand
7.0	11.3	14.0
(2.0)	(3.2)	(4.0)
(2.0)	(5.0)	(7.0)

(Subject M. N.)

left hand	right hand →	both hands
26.7	32.8	39.8
average 29.7		
(1.0)		(1.3)
(1.0)	(1.0)	(2.0)

(Subject K. K.)

right foot	left hand →	left hand & right foot
27.6	21.6	19.5
(1.0)	(0.8)	(0.7)
(1.0)	(0.6)	(1.6)

Figures in parenthesis are relative values.

It was found that almost in proportion to the size of the area of the skin exposed to cold was greater the cardiac response. This is the spatial summation.

When the component parts were immeersed successively, however, one after another with a time interval of 3 minutes, there was indeed a summation of effects, but the total cumulative effect came out to be 45 to 60 per cent. of the simultaneous summative effect (cf. Tab. I, b). By the way it was a fact interesting to note that when two parts of extremities distant

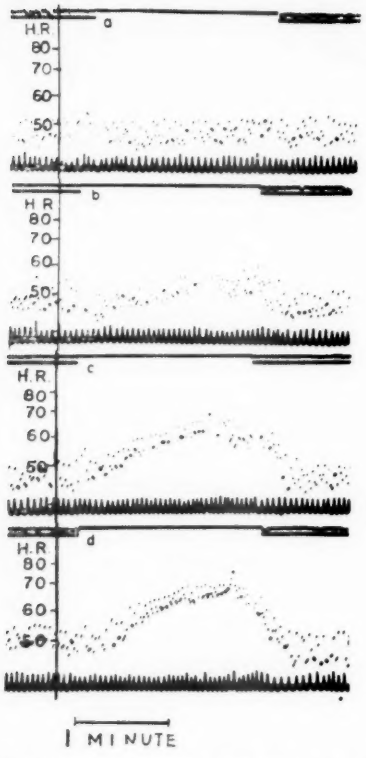

Fig. 7. Cardiotachogram. Spatial summation in cardiac response to cold stimulus .

Upper: Time scale in two second interval.

Middle: Cardiotachogram (its scale in pulse rate per minute is shown on the left).

Lower: Respiratory record, the upward movement is inspration. a: Hand was immersed in water at 37°C for two minutes. b: One finger was immersed in water at 5°C. c: Three fingers were immersed in water at 5°C. d: Four fingers were immersed in water at 5°C.

each other, such as both hands or a hand and the contralateral food were involved, the sensation of pain which had been felt in the first immersed part disappeared as soon as the second part was immersed while the pain and other sensations in the latter part behaved as usual.

Discussion

The results of the present investigation clearly indicate that immer-

sion of the hand or foot in cold water at 12°C or below induces a cardiac acceleration, rise in blood pressure concomitantly with sensation of pain. That the cardiac response is not dependent on the cold sensation *per se* may be inferred from the following facts: (i) Hensel & Zottermann[8] found in the lingual nerve of the cat there were persistent discharges of a single cold fiber even at constant temperatures between 15 and 40°C. According to Dodt's observation[9] the lowest temperature at which the discharge of a single cold fiber ceases lies usually between 10 and 12°C. (ii) In the present study immersion of the hand in cold water above 12°C was found to arouse sensation of cold but no acceleration of the heart. It is to be noted that in this case no sensation of pain was experienced. (iii) Submersion of the body in water of 20 to 25°C caused a drop rather than an increase of cardiac rate in a normal person (Wells, Tuttle & Templin[10]).

It has long been noticed that pain sensation is induced by extreme cooling of the skin. The highest bath temperature at which the cold pain can be produced was found to be 18°C by Wolff & Hardy[4] and about 10°C by Greenfield & Shepherd[11]. The former authors considered this pain was mediated by small non-myelinated C-fiber. Such a pain was also confirmed to occur in the present experiments at temperatures below 12°C. Wolff & Hardy supposed such " cold pain " was caused by the thermal gradient in the hand, while Greenfield & Shepherd[11] considered it attributable to the absolute value of temperature so low as 5°C or below. It can not be decided now which of these is correct.

While Hines & Brown's " cold pressor test " has been well known and widely used as a measure of vascular reactivity in general, particularly to detect a disposition for essential hypertension, Wolff[5] was led to the conclusion that this phenomenon was rather dependent upon the individual tendency for cold pain than the cardiovascular reactivity, on the basis of his experience about the patients who had lost pain sensation due to organic or functional lesions. Wolff & Hardy[4] proved in healthy men that the pressor response and severity of cold pain are both roughly proportional to the degree of local cooling. The present study indicates intimate relationship between the cardiac response and the pain.

It seems strange, however, that in the experiments described above the cardiac acceleration appeared always before the sensation of pain (Figs. 2, 5 & 6). It may be that the sudden set-in of cold sensation upon immersing the hand is responsible for the initial phase of cardiac acceleration.

The possible relationship between the cardiac response and the cold pain can be recognized from the simultaneous occurrence of adaptation of both. Such an adaptation in cold pain has been reported by Wolff & Hardy[4] and Greenfield & Shepherd[11]. Dodt[9] found in the cat that

sudden cooling of the tongue elicited a *brief* but intense discharge in cold fibers of the lingual nerve.

Greenfield & Shepherd[11] considered the adaptation was due to the augmentation of blood flow to the cooled area because of the cardiovascular reaction to cold which had been confirmed by several investigators[12–15]. In the present study it was ascertained that the adaptation did not occur as long as the blood supply to the cooled part was occluded, thus verifying the idea just mentioned above.

In view of these evidences it may be inferred that the changes in cardiac rate and blood pressure as well as respiratory movement are due to the reflex mediated by centripetal pain fibers. The impulses produced in the latter by local cooling are on the one hand conveyed to the sensory cortex of the brain to cause sensation of pain, and on the other hand to the cardiovascular and respiratory centers to exert its effects at the respective end-organs.

As described the degree of cardiac rate increase is a negative linear function of the water temperature. A similar, logarithmic relationship was observed in the rabbit by Hoshi *et al.*[2] between the strength of chemical stimulus applied to the nasal mucosa and the extent of reflex cardiac slowing. Hartline[16] found that the frequency of spikes in the optic nerve stands in the same functional relationship to the intensity of light stimulus. The logarithmic law is in accord with the Fechner's law concerning stimulus-response relationship in general. In the cold-immersion experiments the pain fibers would have been conveying impulses in frequencies in a linear function to the logarithms of water temperature.

Now the question arises whether the effects of cooling are summative or not. Sherrington[17] provided evidences that in spinal reflex the effects of noxious stimulus from an adjacent body areas are summated at the segmental level spatically as well as temporally. Hardy *et al.*[18] also recognized spatial summation in some motor response to pain, though they failed to confirm this property as to the sensation of pain produced by cooling. The results of the present study indicate the existence of spatial summation of reflex effects of local cooling. When more than two portions of hands or feet were exposed to cold one after another, the total effect was found to be more or less milder than when they were cooled at the same time probably because adaptation of pain reflex from the area stimulated early would have in effect when other parts came to be stimulated by cold (cf.[4] p. 135).

Summary

1. Changes in cardiac rate and blood pressure when the hand or foot was immersed in cold water was investigated in human subjects.

2. When the water temperature was above 12°C no change in cardiac rate was observed in spite of the subjects' distinct feeling of cold. At temperatures below 12°C the heart became accelerated, along with a rise of blood pressure and respiratory rate. The cardiac rate, after it had attained its peak value in 60 to 90 seconds of immersion, showed a tendency to return gradually to its original rate notwithstanding that the hand was remaining in cold water (adaptation).

3. In cases when the water temperature was below 12°C, the increase in cardiac rate changed in a linear function with the logarithm of water temperature. It is also proportional to the area of the skin exposed to cold, or to the sum of areas when several parts of hands or feet were cooled simultaneously (spatial summation).

4. With the temperature of water sufficiently low, an aching sensation (so-called " cold pain ") was aroused shortly after immersion beside the sensation of cold. This pain also tended to subside spontaneously after a few minutes. Almost at the same time and in the same pace there took place the subsidence of increase in heart rate, of rise in blood pressure as described above, concomitantly with a slight rise of skin temperature which had fallen to its minimum value.

5. When the blood circulation to the immersed part was arrested neither adaptation of cold pain nor that of cardiovascular reactions were observed.

6. These facts indicate that the cardiac rate and blood pressure changes are reflexes brought about by local cooling, for which predominantly the sensation of pain rather than cold is responsible, and suggest that the phenomena of adaptation in cardiovascular response are probably based on a common factor, the adaptation of cold pain. The cause of the latter may be sought in a possible increase of peripheral blood flow as a reaction to local cooling.

Acknowledgement

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